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PROXIMATE FACTORS INFLUENCING THE TIMING AND OCCURRENCE OF REPRODUCTION IN RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*)

BY

CONSTANCE DUSTIN BECKER ©

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA
SPRING, 1992

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YOU CAN REMEMBER WHEN,
WHEN THE WORLD, ONCE THE WORLD WAS
ALIVE
A WILDER PLACE,
FAR FROM THE WAYS OF MEN-
I CAN FEEL IT AGAIN.

- Cecelia Ostrow

" Red Squirrel "

Kenneth J Reeve

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Abstract

Red squirrels, *Tamiasciurus hudsonicus*, show more asynchrony in dates of estrus (a two to three month range) than ground squirrels (a two week range), and the cues of estrus in red squirrels are not known. Laboratory and field studies were conducted from November 1987 to July 1990 to test hypotheses about the proximate factors that cue reproductive readiness and influence the timing and probability of estrus and parturition in red squirrels. Research methods included controlled day length experiments, food additions to specific individuals, body weight and fat analyses, and long-term recapture of live squirrels.

Laboratory results suggest that female red squirrels have endogenous circannual rythms that are sensitive to changes in day length. Squirrels living in artificial long-day conditions (>10 h light) entered proestrus (reproductive readiness) one to two months earlier than individuals kept in short-day conditions (< 8 h light). Short day conditions delayed but did not prevent proestrus and estrus from occurring.

Wild squirrels were studied in the conifer forests of the Athabasca Sandhills near Ft. Assiniboine, Alberta. Results of food addition experiments and records of annual patterns of reproduction indicated that differences in net energy gain (the caloric balance between food energy obtained and the costs associated with survivial and obtaining food) and individual sensitivity to net energy gain cause variation in timing and the probability of estrus and parturition in red squirrels. Spring temperatures and fall cone crops determine the net energy gains of squirrels each spring, and interact with intrinsic aspects of the individual to determine timing and extent of reproductive investment. On a local scale, the distribution and abundance of seed bearing trees near each squirrel's food cache can influence the ability to reproduce.

When size and age were taken into consideration, body weight was a good measure of body fat stores in adult red squirrel females. Female red squirrels given sunflower seeds gained weight earlier and bred earlier in the season than control females that relied on extracting seeds from jack pine cones. The " seed addition" females also had a much higher probabiltiy of producing a litter. Logistic regressions indicated that regardless of age, increases in body fat (weight) improved the likelihood of ovulation and litter production in red squirrels. Despite equivalent access to food, and only slight variation in body weights, experimental females showed a month-long range in timing of estrus. This lack of synchrony may be caused by variation among females in the amount of energy required to cue estrus. On the other hand, it may reflect differences in energy allocation to activities other than reproduction.

In control groups of female squirrels, age consistently explained differences in body condition and ability to reproduce. Wild juvenile squirrels rarely had an estrus cycle ($p < 0.15$) and were delayed if they did. In contrast, wild juvenile squirrels that were fed sunflower seeds had a high probability of having an estrus cycle ($p > 0.8$), and cycled as early in the season as multiparous adults. These results show that young females normally fail to meet net energy requirements for reproduction. As they get older, squirrels either learn to maximize net energy gains, or have lower energy requirements. Foraging strategies and cone handling efficiencies seem to change with age and may determine net energy gains.

Several authors have cited years in which squirrel reproduction seems to anticipate a large cone crop: ovulation rates, the proportions of females breeding and litter sizes were larger in a spring following a poor cone crop, but preceding a large one. Results of experiments and literature review suggest that anticipatory fertility in squirrels occurs because of the age structure of the female population. In jack pine (*Pinus banksiana*) habitats, three dietary sources of anticipatory fertility (plant secondary compounds, protein and energy in buds) were refuted. In spruce (*Picea sp.*) habitat, when squirrels rely on buds, anticipatory litter sizes may be caused by increases in dietary protein.

Further research is needed on the photosensitivity of male squirrels and on the ontogeny of foraging and its effect on net energy gain by individual squirrels.

ACKNOWLEDGEMENTS

In an alpine meadow in Switzerland, during the summer of 1987, I sat wondering what I might study in Canada. Jean Lauber and Stan Boutin answered my postcard expressing their willingness to direct a graduate student. Jean was researching light and vertebrate endocrine systems and was interested in an ecological study. Stan was studying population dynamics of boreal mammals, especially red squirrels in the Yukon. The doctoral project evolved out of shared interests and for the last four years both Jean and Stan have helped me with ideas, money and academic guidance at every stage of the work. They questioned, challenged, encouraged, and provided moral support. I can not thank them enough for all of their assistance.

Funding for the red squirrel study came from the Boreal Institute for Northern Studies (now the Canadian Circumpolar Institute), NSERC grants to Stan Boutin and Jean Lauber, NSERC graduate studies fellowships, and the University of Alberta.

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Now that the defense is over, I feel genuine gratitude for the challenges and enjoyable discussions I have had with my committee members: Jean Lauber, Stan Bouting, John Addicott, Keith Denford, and Frank Bronson (external). I thank all of you for reading and commenting on my work.

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I dedicate this thesis to Mark, because he was a major source of inspiration for the project. We decided to come to Canada, and as a consequence, the wildness and beauty of the boreal forests of Alberta and the lives of red squirrels are now part of me. Secondly, I dedicate this thesis to people everywhere who are working to protect wilderness and the wild biotic diversity it holds.

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Chapter 1

Reproduction in *Tamiasciurus hudsonicus (Bangs)* : Why study proximate factors?

We have not studied enough wild mammals in sufficient detail both in the laboratory and in their natural habitats to provide a truly meaningful overview of the environmental control of reproduction in this class of animals as a whole.

- F. H. Bronson, 1989

1.1 Introduction

A breeding season can be defined as the distribution of reproductive events in a population. Like specific aspects of morphology, physiology and behavior, breeding seasons are designed by the forces of natural selection (Baker, 1938). Over evolutionary time, the breedings seasons exhibited by a species should maximize the survival and reproduction of individuals (Darwin, 1865; Lack, 1954; Lincoln & Short, 1980). Success of reproduction depends upon the timing of reproductive investment, either within a season or within an individual's lifetime. Seasonal breeders face a paradox, because ultimate factors, such as availability of sufficient food for lactation, occur too late in the season to be of any use in timing the onset of reproduction. Thus, many animals rely on proximate environmental cues to time reproduction in advance of conditions that support the care and rearing of offspring (Lincoln & Short, 1980; Sadlier, 1969).

Negus and Berger (1987) distinguish between "obligate" and "facultative" breeders depending on whether the breeding season varies in response to environmental variation or not. By their definiton, obligate breeders show little variation in the timing of breeding (i.e. more synchrony) and are more likely to evolve endogenous rhythms "programmed" by reliable, proximate environmental variables (i.e. photoperiod, seasonally limited plant compounds). Obligate breeders survive in relatively predictable environments, whereas facultative breeders have experienced selection for optimal performance in fluctuating environmental conditions (Negus and Berger, 1987). As a consequence, facultative or opportunistic breeders should time their reproductive effort in response to "suites of environmental information". The breeding seasons of mammals are not likely to fit perfectly into these two extreme catagories of obligate or opportunistic, but probably form a continuum. In addition, life history aspects of the mammal, such as life-span, are likely to determine whether breeding may be fine-tuned and obligate or opportunistic (Bronson, 1989).

Few researchers have studied the proximate factors regulating the timing of breeding in wild mammals. Instead, studies have focused on domesticated or laboratory mammals with obligate responses to proximate cues, such as the response of sheep to short days, and hamsters to long days (Bronson, 1989). In studies of obligate breeders, functional physiologists have made great advances towards an understanding of the complex relationships among light perception, melatonin, gonadotropins and the timing of reproduction in mammals (Binkley, 1988). In field studies, the approach for an understanding of variation in the timing of breeding has been one of observational study, rather than experimental work (Bronson, 1989; Ims, 1990).

The present study is one of the first to conduct parallel field and laboratory experiments to identify the proximate environmental factors responsible for timing reproduction in a wild seasonally breeding mammal. Red squirrels are an excellent model species for such study because they are often territorial, fairly long-lived, and typically breed only once in a season. Thus, the same animal can be reliably found in the same place during several breeding seasons. Factors such as food availability or quality can be manipulated to affect individual animals.

Understanding the proximate factors that influence breeding in red squirrels is of interest for a variety of reasons. Most importantly, there are several hypotheses about productivity and the timing of breeding in this species that have not been tested. These are of great interest because by testing them we advance our understanding of mammalian reproduction in general. From an applied point of view, the ability to increase the fecundity of red squirrels may assist with the recovery plan for endangered populations such as the one on Mt. Graham, Arizona. The knowledge might also be used for controlling squirrel productivity so as to reduce the damage the animals do to tree crops.

The broad objectives of this doctoral study are:

1. To determine the proximate annual cues of proestrus and estrus in the red squirrel;
2. To formulate and test hypotheses about environmental factors that may cause variation in timing of estrus and parturition in squirrels;
3. To determine the proximate factors that cause variation in fertility, and in particular, the extent of annual primiparity (production of the first litter) .

In this chapter, I first review the natural history of red squirrels, especially aspects of life history and breeding seasons. Next, the hypotheses and predictions that became the focus of a three year field and laboratory study of red squirrels in central Alberta, Canada are discussed.

1.2 Natural history of red squirrels

The genus *Tamiasciurus* is represented by two species, *hudsonicus* and *douglasii*, both of which inhabit coniferous forests of North America. The two tree squirrel species are very similar in size, foods utilized, and behaviour. In areas of sympatry, they utilize different conifer species (C. Smith, 1970; 1981). Both squirrel species are thought to have evolved from a common ancestor via reproductive isolation during the Pleistocene epoch

(Lindsay, 1981). Today most of the 25 subspecies of *hudsonicus* live in forests east of the Rocky Mountains, while *douglasii* subspecies live to the west of this geographical feature (Gurnell, 1987). In North America and throughout this thesis, *T. hudsonicus* are referred to as red squirrels, and should not be confused with the European red squirrel (*Sciurus vulgaris*).

In mature boreal and temperate coniferous forests, red and Douglas squirrels are territorial. They defend areas that vary in size from 0.25 ha to 1.0 ha depending on the food supply associated with the conifer forest in which they are residing (C. Smith, 1968). Territories vary in quality and in their ability to sustain a squirrel during the winter (C. Smith, 1968; Gurnell, 1984; Kemp and Keith, 1970). In forests with a heterogeneous tree distribution, marginal areas may be abandoned or incorporated into a squirrel's territory. In contrast, "prime" or permanent territories are defended year round (Kemp & Keith, 1970; Rothwell, 1977).

Territorial boundaries vary in shape and distribution (Gurnell, 1984), but all permanent territories contain a major cache of cones and other foods. Commonly called "middens", these food caches are extremely important to squirrels during the winter. The middens are composed of tunnels and storage areas formed in piles of cone bracts that have accumulated during "central-place" feeding bouts. Several underground nests connected by a maze of tunnels leading to food caches and multiple exit-entry holes are typical. These "archaeological sites" are used by many generations of squirrels (Klugh, 1927).

Like other holarctic tree squirrels, the life span of a red squirrel is probably between five and ten years (Gurnell, 1984). Mortality is thought to be relatively high during the first year, with estimates of over 60% in several studies (Kemp and Keith, 1970; Rusch and Reeder, 1978). According to one review of several studies, mortality shows a fairly steady annual decline (average of 20% per annum) with age (Gurnell, 1984).

The life of a young red squirrel begins on its mother's territory in a nest, typically with one to three other siblings. Born in a drey (tree nest), a hollow log or an underground nest (Lair, 1984), the altricial infants depend on maternal care and defense for six to eight weeks (Ferron,1984). At this age, the young emerge from the nest with many of the behaviours typical of adult squirrels, such as foot stamping, cheek-rubbing, tooth chattering and nest building (Ferron, 1981; 1984). Just before weaning, mother squirrels may move their nestlings to the periphery of the territory, where the young "practice" setting up territories (C. Smith, 1968). Curtailment of the mother-infant relationship varies a great deal (Lair, 1984). Some female squirrels give up part or all of their territory to one of their offsping (Price *et al.*, 1986). In other cases the mother aggressively forces the juveniles to disperse from the natal area (Lair, 1984). This range in natal philopatry can even occur within a single study area (Wells, 1987).

The mother-infant conflicts and "decisions" over space (Lair, 1984; C. Smith, 1968) hint at the importance of obtaining a territory. Kemp and Keith (1970) noted that in patchy conifer/deciduous forests juveniles set up "transient" territories in deciduous areas, caching hazel nuts, while adults hold permanent territories in coniferous areas. They found that juvenile mortality (67%) was substantially higher than adult mortality (21-34%), and was influenced partially by the size of the hazel nut crop. Studies in the Yukon, where juvenile mortality in "optimal" areas was 51-69%, have verified further the importance of obtaining a territory in a suitable habitat. In sub-optimal habitats, juvenile mortality may be as high as 100% (Boutin, pers. comm.).

The time of birth may affect a juvenile squirrel's chance of obtaining a territory, and consequently may determine its likelihood of survival. Price (1989) found that juveniles born relatively late in the season were less successful at acquiring a territory away from their natal one. Gurnell (1983) and Rusch and Reeder (1978) found that juveniles born early in the season had better over-winter survival than those born late in the season.

Koford (1979) and Searing (1975) found that food abundance on the territory (in terms of stored seed or density of seed-bearing trees) determined over-winter survival. In combination, these findings suggest that female squirrels producing young early in the season should have higher fitness than those producing litters late in the season. But what determines when a squirrel is born? In a given year, what determines whether a female gives birth or not? These are two questions this study attempts to answer.

1.3 Previous studies on red squirrel breeding seasons

It is well-documented that the proportion of females producing litters varies in the same red squirrel population from year to year (Rusch & Reeder, 1978). However, the factors that cause this variation have not been well-studied. When data from several populations are compared (Table I-1), the maximum variation in the percentage of females breeding from one year to the next is as high as 50%.

Table I-1: Variation in proportions of lactating red squirrels. "% Max" refers to the highest percentage of females breeding in a given season, while "% Min" is the value for a year with the lowest proportion of females breeding.

Location	% Max (N)	% Min (N)	Authors
Alberta	88 (39)	67 (43)	Kemp & Keith 1970
B.C.	83	32	C. Smith 1968
Alberta	96 (105)	72 (112)	Rusch & Reeder 1978
Colorado	89 (27)	59 (39)	Dolbeer 1973

A major source of annual variation in the proportion of breeding females is caused by primiparous yearlings (yearlings producing a litter for the first time). Rusch and Reeder (1978) found that the proportion of primiparous yearlings varied from 24% to 88% in squirrel populations of central Alberta. Most authors speculate that the variation in the proportion of squirrels breeding in a given year is caused by either weather or the size of annual cone crops (Kemp and Keith, 1970; Rusch & Reeder, 1978; Millar, 1968). However, no one has systematically studied variation in primiparity or proportions of breeding females and the proximate environmental factors that influence them.

Compared to hibernating sciurids that have been found to have circannual endogenous rhythms (Kenagy, 1980) and fairly synchronous breeding seasons (two weeks), red squirrels (and other tree squirrels) show remarkable asynchrony in the timing of estrus, conception, and consequent parturition. Rusch and Reeder (1978) documented as much as a two month difference between years in the onset of breeding in the same population. Within a year, the time interval between the earliest red squirrel estrus and pregnancy and the latest may also span two to three months. Rusch and Reeder (1978) also discovered that the percentage of yearlings breeding in a season was correlated with the date of onset of breeding, and that yearlings bred later than adult female squirrels. They concluded that onset of breeding, and the proportion of yearlings breeding in a given year were linked to the length of winter (days of snow cover after January 1) and the size of the fall cone crop. However, no hypotheses were proposed and tested to search for mechanisms underpinning these descriptive patterns.

1.4 Proximate factors affecting reproduction: hypotheses and predictions

The proximate cues used by a facultative breeder are likely to be a "messy", interdependent set of environmental conditions that act to permit or negate reproduction. - Negus and Berger, 1987

Figure I-1 illustrates some of the major potential factors that may influence the timing and occurrence of reproduction (estrus, ovulation, conception and parturition) in a red

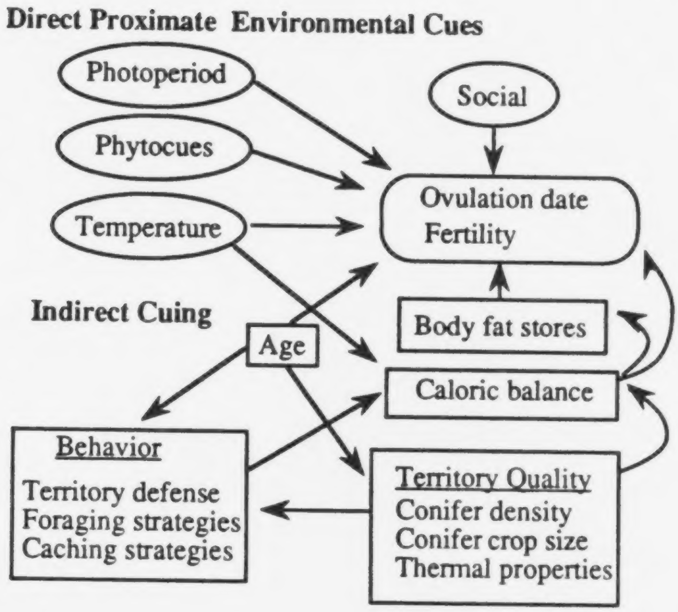

Figure I-1. Potential proximate factors influencing the timing and probability of reproduction in red squirrels.

squirrel female. These include photoperiod, temperature, and plant secondary compounds as potential direct environmental cues. Social cues are another type of direct cue that may trigger reproductive events. As indicated by the arrows in Figure I-1, indirect cuing may involve combinations of factors. For example, a squirrel's body size may influence foraging efficiency, which may in turn influence energy balance and body fat, which may determine timing of estrus and the ability to produce a litter. Likewise, food intake rates may depend upon territory quality and size, foraging behavior and annual seed production by conifers.

Breeding seasons of red squirrels can be divided into several chronological stages:

1. The transition from sexual quiescence to reproductive readiness: testicular recrudescence in males and proestrus in females.
2. The 2-3 day period of estrus, ovulation and mating.
3. Gestation and parturition.
4. Lactation and weaning

The approach of the present research was to determine through experiment and observation the proximate factors suspected to influence female reproduction during the first three stages of the breeding season. Male reproductive readiness was the topic of only one preliminary experiment.

1.4.1 Reproductive readiness: Is photoperiod a proximate cue for timing reproductive readiness in red squirrels?

In studies of wild red squirrel populations, onset of reproductive readiness in females is typically recorded after late January, with most cases occurring in February and early March (Layne, 1954; Dolbeer, 1973; Lair, 1984). Occurring as much as a month before estrus, the vulva swelling associated with proestrus (reproductive readiness) happens when days increase in length in temperate North America. Thus, photoperiod may act as a cue to initiate the physiological changes associated with proestrus, and may also prevent initiation of these endocrine events. Alternatively, the start of breeding could be endogenously set, regardless of light cues available in the spring. On the other hand, the start of breeding could be endogenous, but entrainable by light cues. If squirrels have purely endogenous biological clocks, they should show no change in the timing or occurrence of testes recrudescence or proestrus when day length cues are absent or altered (Kenagy, 1980; 1981). If, on the other hand, red squirrels are photosensitive, the timing of reproductive readiness should be altered by the presence or absence of day length cues.

This thesis describes several experiments designed to test the following hypotheses:

1. Seasonal changes in photoperiod affect red squirrel reproductive physiology.
2. Short days are a prerequisite for testicular recrudescence.

    2a. Hypothesis: The absence of exposure to short day conditions will prevent recrudescence in male red squirrels. (Male squirrels normally become reproductively capable during short day conditions (<10 hrs of light)).

3. Increasing day lengths or long days (>10 hrs of light) are required for reproductive readiness in female red squirrels.

    3a. Hypothesis: Female squirrels kept under continuous short day photoperiods (7 hours) will not show the same physical signs of reproductive readiness (vulva swelling and proestrus vaginal smears) as squirrels exposed to an increasing photoperiod that mimics spring.

    3b. Hypothesis: When exposed to increasing photoperiods in November, female red squirrels will come into proestrus and estrus earlier than females that experience increasing photoperiods in January and February.

The experiments concerned with photosensitivity and reproductive readiness were by necessity laboratory based.

1.4.2 Ovulation & Conception: What determines timing and occurrence?

Asynchrony of ovulation and consequent conception in a season and in different years indicates that the exact timing of ovulation in red squirrels is neither rigidly fixed by endogenous cycles, nor dependent on single-factor environmental cues, such as photoperiod. If an endogenous circannual rhythm exists, it seems to be responsive to and modifiable by environmental conditions that vary from year to year.

Most of the species-specific hypotheses proposed in the literature to explain the variable breeding schedule of squirrels are either food or weather-based (Lair, 1985). First, and most cited, is the "over-winter energy" hypothesis. It supposes a cause and effect relationship between cone crop size and squirrel productivity (Millar, 1970, Searing, 1975), stating that a large fall cone crop will be followed by early breeding and high squirrel productivity. Rusch and Reeder (1978), Millar (1970), and Searing (1975) all reported increased fecundity and early breeding following a cone crop that was relatively large. The "over-winter energy" hypothesis includes the idea that the severity of the winter will influence the food energy available to a squirrel for over-wintering (Kemp and Keith, 1970; Rusch and Reeder, 1978; this study).

C. Smith (1968), Kemp and Keith (1970) and Kelly (1978) had results that conflicted with the "over-winter energy" hypothesis. In years following "light" cone crops, but preceeding "heavy" crops, they found larger litters and a higher percentage of breeding females in their study populations of red squirrels. Kemp and Keith (1970) suggested that the quality of food in spring, especially "nutritionally superior" flower buds (Kramer & Kozlowski, 1960) of spruce could stimulate squirrel reproduction in advance of a high cone crop. This idea was coined the "anticipation" hypothesis.

A third proposal refered to as the "snow-melt hypothesis" by Lair (1985) suggested that onset of breeding depended upon the absence of snow. Rusch and Reeder (1978) found a positive correlation between date of estrus and the number of days with snow on the ground after January 1. They speculated that patches devoid of snow were required for courtship behavior in red squirrels.

Observations of squirrels breeding when snow completely covered the ground negated the snow-melt hypothesis (Lair, 1985). Lair suggested that snow-melt was correlated with thawing of the ground and could be indirectly related to onset of breeding in red squirrels, because temperate zone trees increase bud growth in response to soil moisture and increasing day length. Given that squirrels feed on these parts of conifers, Lair (1985) reasoned that nutrient changes in conifer buds could provide a reliable indicator of the beginning of the annual plant growth cycle regardless of whether it started early or late in a given year. Like Kemp and Keith (1970), Lair (1985) contended that onset and extent of breeding in squirrels could hinge upon nutrients available in buds in early spring. She did not commit herself to assuming that these buds differed in quality before a large cone crop. Lair's idea may be combined with anticipation under the broad category of hypotheses that propose nutrients in buds rather than caloric balances as cues for timing estrus or determining the extent of annual reproduction.

Bronson (1985) suggested that annual patterns of breeding reflect the caloric costs of the female's reproductive effort as they relate to the costs and gains associated with her foraging effort. By combining Bronson's idea with the over-winter energy hypothesis , "the net energy gain" concept evolved: average timing of estrus, asynchrony in reproduction and variation in fertility from year to year are determined by net energy gain to individual squirrels during the breeding season. Net energy gain is defined as the caloric balance between food energy obtained by an individual and the costs associated with daily survival. Net energy gain may be increased by lowered foraging costs, mild weather, or a change in diet. When net energy gain is positive, squirrels should lay down fat stores and gain weight. When net energy gain is negative, squirrels should lose fat and weight. The main exclusive alternatives to this hypothesis are (1) that the variation in reproductive events in squirrel breeding seasons are endogenously pre-programmed or (2) that nutrient cues (other than energy relationships) in the squirrels' diet cause the variation in reproduction. The above hypotheses and their predictions are outlined below.

1.4.2.1 Hypotheses about average timing and probability of estrus and parturition

Hypothesis 1. The average timing of estrus and extent of reproduction depends upon net energy gains in early spring.

Predictions:

a. Estrous dates should be earliest and probability of producing a litter highest in years having low metabolic costs and/or high gains. Late dates of estrus and a low percentage of breeding should be noted under the opposite environmental conditions. Specifically, if winter and spring conditions are mild and follow a peak cone crop, estrus dates should be relatively early and the proportion of females producing litters should be relatively high. Low food abundance combined with low temperatures in spring should cause squirrels to breed later in the year and should reduce the number of individuals producing litters.

b. Additional food should advance estrus and increase the probability of estrus and parturition in female squirrels.

c. Individuals that have high metabolic costs should breed late or not at all. For example, juveniles may fail to breed or breed late in the season because:

1. Juveniles are more limited by food resources than adults because they are still growing during the winter of their first year.
2. Food intake is limited by the fact that juveniles are small squirrels.
3. Food intake is limited by the fact that juveniles are less experienced than adults.

4. Some squirrels may fail to breed because of energetic costs associated with territory quality.

4a. Hypothesis: Territories used by successful breeders should differ from those of non-breeding females in terms of harvesting or foraging costs.

1.4.2.2 Hypotheses about the causes of asynchrony in timing of estrus in red squirrels

At least three potential proximate mechanisms may be tested to explain the relatively large asynchrony or "spread" in estrus dates exhibited by squirrels during a breeding season. The first idea is based on the net energy gain concept, while the other two are alternative hypotheses.

1. Female squirrels may differ in the amount of food energy available to them during the breeding season.

   1a. Hypothesis: If variation in net energy availability causes asynchrony, then food addition should synchronize estrus dates during a season.

2. Female squirrels vary in sensitivity to energetic costs and gains, and these individual traits (possibly genetic) cause asynchrony.

   2a. Hypothesis: If individual variation is a factor, females should show consistency in timing of estrus from year to year (i.e. Females that breed early in one season should always breed early; and extra food should not synchronize estrus dates)

3. Asynchrony is mainly caused by age differences of females breeding in a given season.

   3a. Hypothesis: Because yearlings breed later in the season than older females (Rusch and Reeder, 1978) their presence will elongate breeding seasons. The more primiparous females there are in a breeding season, the greater the asynchrony and range in estrus dates.

4. Timing of breeding is rigidly set by circannual endogenous endocrine cycles.

4a. Hypothesis: Food addition or quantity should not change the distribution or mean of estrus dates.

1.4.2.3 The potential role of body condition in the net energy gain concept

As a result of variation in net energy gain, body condition may vary in female squirrels. A positive energy balance that favors fat storage may determine the timing and probability of reproduction. Wauters and Dhondt (1989) found such weight thresholds in European red squirrels (*Sciurus vulgaris*). Food-addition should allow individual squirrels to surpass energetic thresholds earlier in the season, thus advancing ovulation and conception. If this is the case, early weight gain in spring (positive energy balance) should correlate with early estrus and births.

In a recent review, Bronson and Manning (1991) have compiled evidence for and against the idea that ovulation in mammals is dependent upon a critical amount of body fat. Although this study does not employ detailed metabolic studies, it does attempt to measure body fat in squirrels and determine relationships between these fat levels and probability of estrus and parturition.

1.4.2.4 Further tests for nutrient cues and anticipation

I wanted to explore further the source of the anticipatory fertility reported by Kemp and Keith (1970). Could anticipation be explained by net energy gain or other processes than those proposed by Kemp and Keith (1970) and reiterated by Lair (1985)? The "anticipation hypothesis" suggests that either energy, nutrients, or a special compound in conifer buds influences ovulatory rate or the survival of embryos, such that more squirrels reproduce prior to a high mast crop. I tried to refute the net energy gain hypothesis, explored three dietary components that might cause anticipation and searched for other reasonable explanations for the apparent anticipation (Chapter 7).

1.4.3 Potential for social cues in red squirrel reproduction

For many mammals there is strong evidence that social cues modulate the timing of breeding. For example, the timing of first ovulation in house mice, *Mus musculus*, depends on the female's social environment. In many rodents, pheromones produced by sexually active males accelerate ovulation, while cues from other females prevent it (Vandenberg, 1983). The importance of social interactions cannot be ignored. However, in red squirrels, a solitary territorial mammal that has spontaneous ovulation, social cuing is unlikely to play a major role in determining the start of the breeding season or the actual date of estrus.

As mentioned earlier, red squirrels in northwestern coniferous forests exclude conspecifics from their territories year-round except during the brief one to three day ovulatory phase when mating chases occur (C. Smith, 1968; Price *et al.*, 1986; this study). Even in areas with overlapping home ranges, in the eastern deciduous forests, little social contact occurs among adult red squirrels (Lair, 1985). Female red squirrels ovulate in the absence of male squirrels (Modafferi, 1972), so pheromones and tactile stimulation are not required for onset of proestrus, estrus and ovulation. Choice of a mate by a female red squirrel and consequent conception may be affected by social cues. However, these topics are beyond the intended scope of this study.

1.5 Organization of the thesis

The following chapter describes general methods and the field study site near Fort Assiniboine, Alberta. Laboratory studies on the role of photoperiod in the timing of reproductive events of red squirrels are presented in Chapter 3. That chapter reports on the effects of short and long days on both male and female reproductive readiness. I report measurements of testicular recrudescence in males and proestrus in females.

Because body weight has been shown to be important for determining onset of estrus and fertility in other mammals (e.g. squirrels and red deer; see Wauters & Dhondt, 1989), it is important to understand the factors influencing body weight in red squirrels. Chapter 4 describes the relationships between body weight, fat stores, age, and reproductive history of female squirrels in different seasons. That chapter describes and assesses a method for measuring body fat in live animals using TOBEC (total body electrical conductivity).

Chapters 5 & 6 focus on the role of food availability and bioenergetic costs of obtaining food energy as proximate factors in determining the timing of estrus, and on the probability of estrus and successful parturition in female red squirrels. Chapter 5 describes the effects of food addition on weight, and body fat and how these changes are related to the timing of estrus. The following chapter describes the effects of food-addition on the proportions of females breeding in different reproductive and age classes. This chapter also examines the relationship between territory quality and breeding performance.

Chapter 7 reviews Kemp and Keith's (1970) "anticipation" hypothesis, which suggests that red squirrels respond to nutrients in tree buds and that these modify squirrel fecundity in advance of a large cone crop. Three possible dietary mechanisms of anticipation are proposed and tested in squirrels and rats: secondary compounds, protein levels and caloric content of buds.

The last chapter synthesizes and summarizes the discoveries made during this research. Topics requiring further study are suggested.

1.6 Literature Cited

Baker, J. R. 1938. The Evolution of breeding seasons. In: *Evolution: Essays on Aspects of Evolutionary Biology*, G. R. de Beer (ed.), Oxford University Press, London, 161-177.

Binkley, S. 1988. *The Pineal*. Prentice-Hall. New Jersey.

Bronson, F. H. 1989. *Mammalian Reproductive Biology*. University of Chicago Press,Chicago.

Bronson, F. H. 1985. Mammalian reproduction: an ecological perspective. *Biol. Reprod.* 32: 1-26.

Bronson, F. H. and J. M. Manning. 1991. The energetic regulation of ovulation: a realistic role for body fat. *Biol. Reprod.* 44: 945-950.

Darwin, C. 1859. *The Origin of Species by Means of Natural Selection*, 1st ed. John Murray, London.

Dolbeer, R. A. 1973. Reproduction in the red squirrel (*Tamiasciurus hudsonicus*) in Colorado. *J. Mamm.* 54(2): 536-540.

Ferron, J. 1981. Comparative ontogeny of behaviour in four species of squirrels (Sciuridae). *Z. Tierpsychol.* 55:193-216.

Ferron, J. 1984. Behavioral ontology analysis of sciurid rodents, with emphasis on the social behaviour of ground squirrels. In: J. O. Murie and G. R. Michener (eds.), *The Biology of Ground Dwelling Squirrels*. University of Nebraska Press, Lincoln, 24-42.

Gurnell, J. 1987. *The Natural History of Squirrels*. Christopher Helm, London.

Gurnell, J. 1984. Home range, territoriality, caching behaviour and food supply of the red squirrel (*Tamiasciurus hudsonicus fremonti*) in a subalpine lodgepole pine forest. *Anim. Behav.* 32: 11191-1131.

Gurnell, J. 1983. Squirrel numbers and the abundance of tree seeds. *Mammal Rev.* 13: 133-148.

Ims, R. A. 1990. The ecology and evolution of reproductive synchrony. *Trends in Ecology and Evolution* 5: 135-140.

Kelly, D. G. 1978. *Population Density, Territoriality and Foraging Ecology of Red Squirrels (Tamiasciurus hudsonicus) in Black and White Spruce Forests of Interior Alaska.* M.S. thesis. University of Alaska, Fairbanks, Alaska.

Kemp, G. A. and L. B. Keith. 1970. Dynamics and regulation of red squirrel (*Tamiasciurus hudsonicus*) populations. *Ecology* 51(5): 763-779.

Kenagy, G. J. 1980. Interrelation of endogenous annual rhythms of reproduction and hibernation in the golden-mantled ground squirrel. *J. Comp. Physiol.* 135:333-39.

Kenagy, G. J. 1981. Endogenous annual rhythm of reproductive function in the non-hibernating desert ground-squirrel *Ammospermophilis leucurus*. *J. Comp. Physiol.* 142: 251-58.

Koford, R. R. 1979. *Behavior and Ecology of a California Population of T. douglasii.* Ph. D. Thesis. University of California. Berkeley.

Kramer, P. J. and T. T. Kozlowski. 1960. *Physiology of Trees.* McGraw-Hill Book Co. Inc., New York.

Labov, J. B. 1977. Phytoestrogens and mammalian reproduction. *Comp. Biochem. Physiol.* 57(A): 3-9.

Lack, D. 1954. *The Natural Regulation of Animal Numbers.* Clarendon Press, Oxford.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Lair, H. 1984. *Adaptations de l'ecureuil roux (Tamiasciurus hudsonicus) a la foret mixte coniferes-feuillus: impact sur l'ecologie et le comportement des femelles reproductrices.* Ph. D. thesis. De L'Universite Laval. Quebec.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Lincoln, G. A. and R. V. Short. 1980. Seasonal breeding: nature's contraceptive. *Rec. Prog. Horm. Res.* 36:1-52.

Lindsay, S. L. 1981. Taxonomic and biogeographic relationships of the Baja Californian chickarees (*Tamiasciurus*). *J. Mamm.* 62: 673-82.

Millar, J. S. 1970. Variations in fecundity of the red squirrel, *Tamiasciurus hudsonicus* (Erxleben). *Can. J. Zool.* 48: 1055-1058.

Millar, J. S. 1968. *The Reproductive Biology of the Western Red Squirrel.* Ph. D. thesis. University of British Columbia. Vancouver, B. C.

Modafferi, M. M. 1972. *Aspects of Reproductive Biology of the Red Squirrel (Tamiasciurus hudsonicus).* M.S. thesis. University of Alaska. Fairbanks, Alaska.

Negus, N. C. and P. J. Berger. 1987. Mammalian reproductive physiology: adaptive responses to changing environments. In: *Current Mammalogy.* New York, Plenum Press. 149-173.

Price, K. 1989. *Territorial Defense and Bequeathal by Red Squirrel Mothers in the Northern Boreal Forest.* M.S. thesis. University of British Columbia. Vancouver, B. C.

Price, K., K. Broughton, S. Boutin and A. R. E. Sinclair. 1986. Territory size and ownership in red squirrels: response to removals. *Can. J. Zool.* 64: 1144-1147.

Rothwell, R. H. 1977. *The Effects of Forest Type and Mast Crop on Winter Territories of and Population of Red Squirrels (Tamiasciurus hudsonicus baileyii) in the Laramie Range of Southeastern Wyoming*. M.S. thesis. University of Wyoming, Laramie, Wyoming.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

Sadlier, R. M. F. S. 1969. *The Ecology of Reproduction in Wild and Domestic Mammals*. Methuen. London.

Searing, G. F. 1975. *Aggressive Behavior and Population Regulation of Red Squirrels (Tamiasciurus hudsonicus) in Interior Alaska*. M.S. thesis. University of Alaska. Fairbanks, Alaska.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels Tamiasciurus. *Ecol. Monogr.* 38(1): 31-63.

Smith, C. C. 1970. The coevolution of pine squirrels (*Tamiasciurus*) and conifers. *Ecol. Monogr.* 40(3): 349-371.

Smith, C. C. 1981. The indivisible niche of *Tamiasciurus*: an example of nonpartitioning of resources. *Ecol. Monogr.* 51(3): 343-363.

Smith, M. C. 1968. Red squirrel responses to spruce cone failure in interior Alaska. *J. Wildl. Mgmt.* 32(2): 305-317.

Vandenberg, J. G. 1983. Pheromonal regulation of puberty. In: *Pheromones and Reproduction in Mammals*. J. G. Vandenberg (ed.). Academic Press. New York. p. 95-112.

Wauters, L. and A. A. Dhondt. 1989. Body weight, longevity, and reproductive success in red squirrels (*Sciurus vulgaris*). *J. of Anim. Ecol.* 58: 637-651.

Wells, N. M. 1987. *The Dynamics of Space Use in a Red Squirrel, Tamiasciurus hudsonicus Population*. Ph. D. dissertation. University of Michigan. Ann Arbor.

Chapter 2

Study Site and General Methods

2.1 Introduction

The hypotheses mentioned in Chapter 1 were tested in laboratory and field studies conducted from November 1987 to July 1990. Field studies were done in central Alberta mainly in the fall, winter and spring. Laboratory studies were done during the same time of year at the University of Alberta. Statistical analyses were done on IBM and Macintosh personal computers.

Methods for collecting data that were basic to the overall theme of the thesis are given below, whereas specific procedures for fat analyses and food supplementation are included within appropriate chapters. There is some redundancy in the methods described in chapters three through seven because each chapter is written as a paper.

2.2 Field study site and field methods

Field studies were conducted near Fort Assiniboine, Alberta, at the Holmes Crossing area of the Athabasca Sandhills (54° 17' N, 114° 45'W) (Figure II-1). The transverse dunes of the Athabasca River system are unique in North America (David, 1977). Uplands are dominated by stands of jackpine (*Pinus banksiana)*, while spruce-tamarack stands and bogs (*Picea glauca, P. mariana, Larix sp.*) are typical in lowlands. Patches of quaking aspen (*Populus tremuloides*) and paper birch (*Betula papifera*) are also present. At this latitude, the climate is extremely seasonal, with below freezing temperatures prevailing from December through mid-March. Figures II-2 a & b depict minimum and maximum daily temperatures that were recorded at the Fort Assiniboine Fire Station from November 1987 to July 1990.

2.2.1 Trapping and definition of age-reproductive classes

Throughout the year, red squirrels were trapped alive on their territories (Price *et al.*, 1986) at their middens. Tomahawk live traps baited with peanut butter were set at each midden and were left open for no longer than 2 hours between checks. A single midden consisting of underground tunnels and stored cones was associated with each squirrel territory. Five middens were excavated in the fall of 1988. They ranged from small (60 cm surface diameter and 30 cm deep) with less than 2,000 cones, to large (five m surface diameter and one m deep) with more than 6,000 cones.

The middens were easy to locate because piles of freshly peeled cone bracts and pine cones stood well above ground level. Some squirrels had more than one midden on a territory, and some kept peripheral piles of cones, but in all cases one midden was largest in size and cone abundance, and was used most often by the squirrel (pers. obs.). Zimmerling (pers. comm.) found that the number of cones piled on the top of a midden is correlated with the number stored underground in a midden.

At first capture, a squirrel was tagged on each ear with a metal tag. The sex of the squirrel was distinguished by looking at the genital region. If male, the genital region was palpated and testes were scored as inguinal or scrotal. When testes were present in the scrotum, their length and width were measured with vernier calipers.

Vulva widths and nipple lengths were measured on female squirrels. Nipple conditions were scored 1-4 depending on length, pigmentation, swelling and presence or absence of milk as summarized in Table II-1.

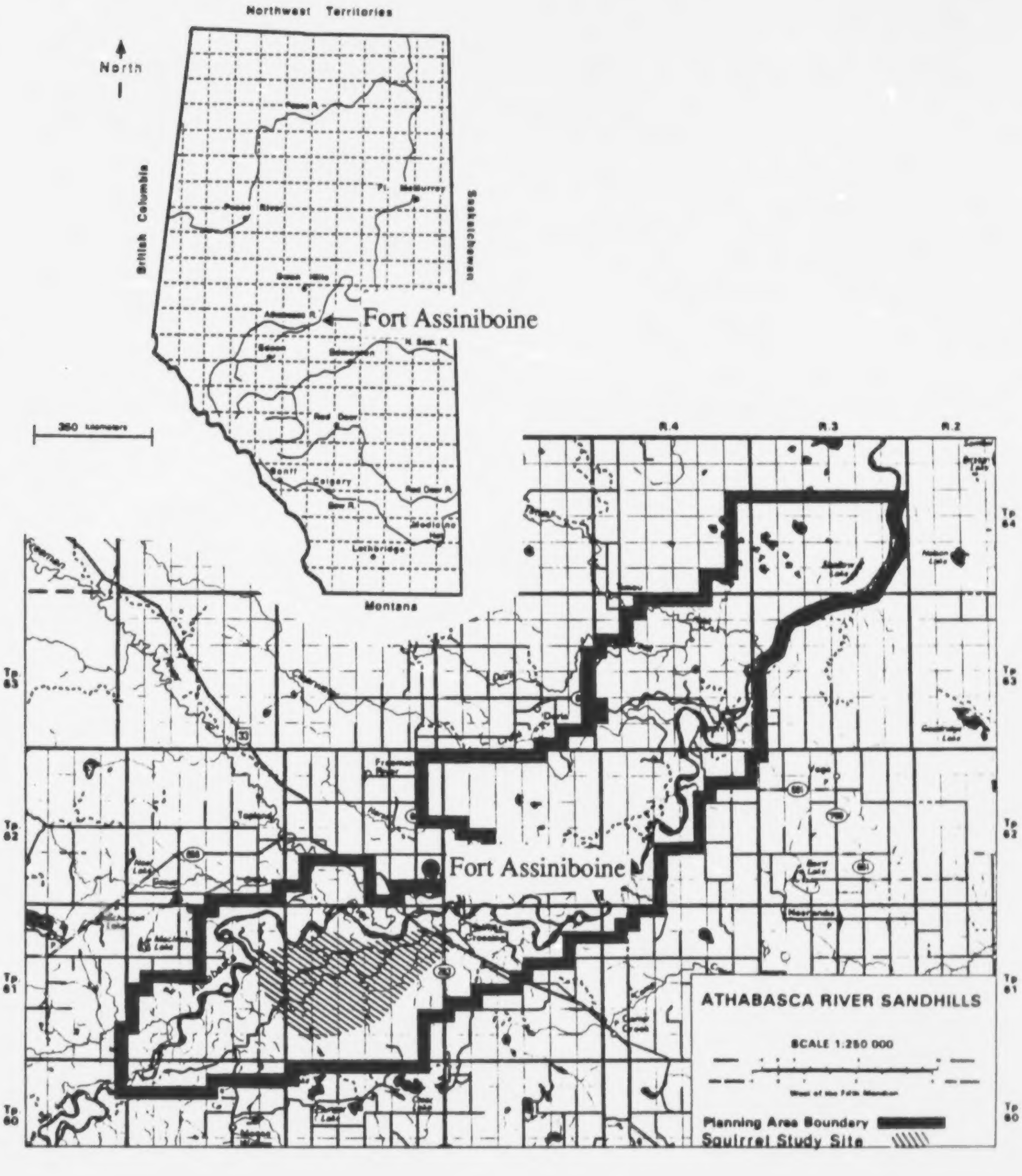

Figure II-1. Map of Alberta showing the general location of the field study site near Fort Assiniboine (above). Township map (1: 250,000) showing the location of the study site in the Athabasca River Sandhills.

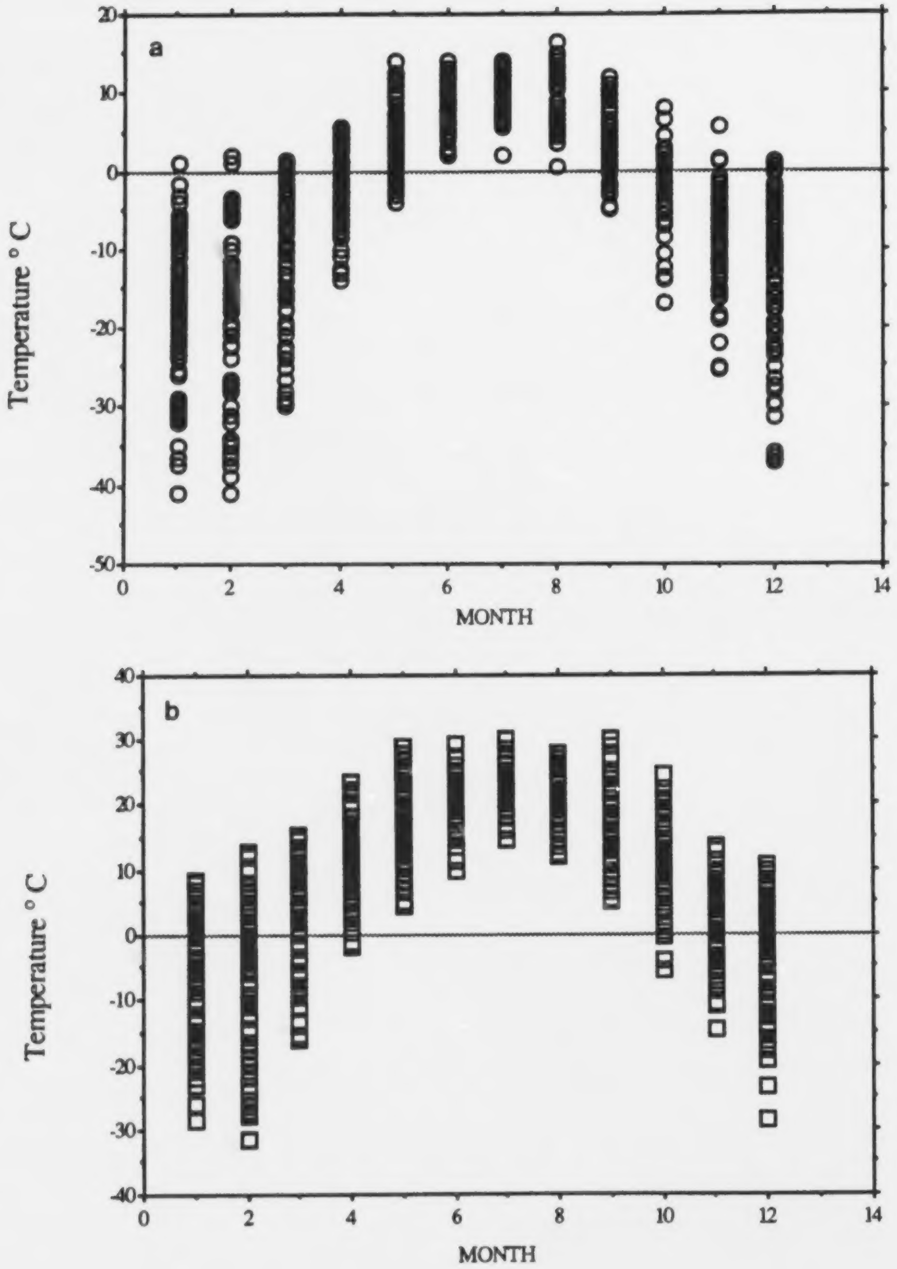

Figure II-2. a. Minimum daily temperatures in centigrade recorded at the Fort Assiniboine forest station (November 1987 - July 1990). b. Maximum daily temperatures in centigrade recorded at the Fort Assiniboine forest station (November 1987 - July 1990). (January = month 1.)

Females were categorized by their reproductive experience and age. Juveniles were easily recognized in the fall by body size, weight, and sparsely furred tails. Nulliparous adults were distinguished from multiparous adults by nipple size and pigmentation (Layne, 1954). Females that had not reached puberty were readily identified by nearly absent, pink nipples, less than 1.0 mm long. Multiparous females had elongated and usually pigmented nipples that were longer than 2.0 mm. Females that cycled but failed to conceive could also be distinguished by pigmented and slightly elongated nipples (1.5 mm). In spring any female that produced a litter for the first time was referred to as a primiparous female. Primiparous juveniles (possibly called yearlings in other articles (Rusch and Reeder, 1978) attained puberty and produced a litter in the spring after they were born (at 11-14 months of age). Primiparous adults produced their first litter during the second or third spring following their birth.

Table II-1. Categories of mammary development in red squirrels

External Nipple Features	Code	Status of Female
Length less than 1.0 mm, pink, no swelling	1	nulliparous
1.0-2.0 mm pigmented, no swelling	1.5	first estrus
Greater than 2.0 mm, pigmented or pale, no swelling	2	multiparous
Greater than 2.0 mm, swollen at base of nipple	3	pregnant
Greater than 2. 0 mm, milk extractable	4	lactating

Anal gland width was measured as an index of scent production. The amount of exudate obtained by squeezing the gland was recorded as none, slight, moderate, or abundant. "Slight" was noted if the gland became moist, "moderate" when a complete drop could be extracted and "abundant" when more than one drop was produced.

During the winter and spring, female squirrels were recaptured at least twice per month. At each capture, weight (grams), size (zygomatic width in mm), and reproductive condition were assessed. After the first signs of proestrus when vulva swelling was noted, the squirrels were trapped more frequently, usually every three to four days, in order to detect dates of estrus and parturition.

2.2.2 Feeding behavior and activity monitoring

The behavior of female squirrels was recorded during 200 hours of focal animal sampling (Altmann, 1974). Tagged females were selected for observation at different times of the day, so that all daylight hours were sampled at least 8 times during fall, winter, spring and summer. Upon finding a female squirrel, 40 to 120 minutes were spent recording the foods eaten and the rate at which various foods were consumed. Every 30 seconds, the squirrel's activity was recorded: resting, feeding, traveling, etc. Time spent in different activities was estimated from the results of these scans (Altmann, 1974). Radio collars were sometimes required to keep track of the animals and were used in about 30% of the sampling periods.

In addition to focal samples, the behaviour of squirrels near their middens was recorded during trap setting sessions. If a squirrel was observed during a trap setting session, its horizontal distance from the midden and height in the forest were recorded along with its activity, the time of day, temperature and date.

As a measure of activity and territorial defense, the number of "rattle" vocalizations made by squirrels (C. Smith, 1968) were recorded on an hourly basis. These data are not presented in this thesis.

2.2.3 Food additions and assumptions about effects

Unlike most studies of rodent populations (i.e. Klenner, 1990; Sullivan, 1990), food was not added randomly or evenly to the whole study population. Instead, individuals varying in age and reproductive experience were randomly selected as recipients of sunflower seed supplements. The exact methods associated with these seed additions are discussed in later chapters (Chapters 4-6).

Sunflower and jack pine seeds have similar caloric and protein content (Table II-2; Appendix II; Gurnell, 1987; this study). However, handling time is far less for a sunflower seed than a jack pine cone. On average, adult red squirrels spent 268 ± 113 seconds (100 observations) to extract the seeds from a single jack pine cone (field observations, this study). To obtain the equivalent seed mass from unshelled sunflower seeds, a squirrel must open and consume two seeds. This takes approximately 20 seconds, a 12-fold decrease in handling and ingestion time compared to jack pine cones.

Due to the reduction in handling time, both caloric and nutrient intake rates are potentially enhanced by a sunflower seed diet. However, because specific minerals, vitamins and proteins are usually required in small quantities relative to calories (Maynard & Loosli, 1969), it was assummed that they are unlikely to be limiting the timing of ovulation in a seed eater. Studies by Havera and Smith (1979) suggest that with the exception of sodium and calcium, seeds satisfy the nutrient and energy requirements of lactating female squirrels. Thus, a seed diet should be sufficient in nutrients to support the less metabolically expensive phases of reproduction: ovulation and gestation. Although sunflower seeds and pine seeds differ in amounts of potassium, sodium and phosphorus, there seem to be no major nutrients that are present in one and not in the other (Table II-2; Appendix II). Thus, I have also assumed that increased fertility (enhanced proportions of animals having and estrus and producing a litter) in groups given sunflower seeds is a consequence of caloric availability, not a response to changes in other nutrients. On the other hand, differences in litter size and infant growth rates may be attributed to a maternal diet that is high in protein and minerals (Pond, 1984).

Table II-2. The nutrient compostion of 1 oz. (28 g) of sunflower and pinyon seeds (Gebhardt & Mathews, 1981) and jack pine seeds (this study)

Nutrient	Sunflower seed	Jack pine seed	Pine seed (pinyons)
Water	5.0%	5.0%	6.0%
Calories	160.0	169.4	160.0
Grams of protein	6.0	6.1	3.0
Grams of fat	14.0	7.4	17.0
Saturated fat (g)	1.5		2.7
Unsaturated fat (g)	12.0		13.8
Carbohydrate (g)	5.0		5.0
Calcium (mg)	33.0		2.0
Phosphorus (mg)	200.0		10.0
Iron (mg)	1.9		0.9
Potassium (mg)	195.0		178.0
Sodium (mg)	1.0		20.0

2.3 Detection of proestrus, estrus and parturition

Some females may enter proestrus but fail to come into estrus and ovulate. Likewise, successful ovulation may not lead to parturition. It is useful to look at the reproductive events of proestrus, estrus and parturition as separate steps along a pathway of reproductive investment that becomes more and more "costly" with each step.

Macroscopic features of the genital region and microscopic analysis of vaginal smears (Zarrow *et al.*, 1964) were used to determine the reproductive state of female squirrels, and have been described by several biologists (Modafferi, 1972; Millar, 1968; Layne, 1954). Macroscopic features associated with all of the reproductive events in red squirrels have also been descriptively correlated with microscopic analysis of cells in vaginal smears (Modafferi, 1972). Proestrus and estrus are easily recognized by increases in vulva width (3.8 - 7.0 mm) and change in epidermal texture and color (Modafferi, 1972; Miller, 1968; Dolbeer, 1973). During proestrus, the vulva changes from being flaccid and pale to turgid and reddened, with maximal widths (5.0-7.0mm) around the time of mating (Dolbeer, 1973). After ovulation, the vulva becomes flaccid, pale and narrow again (2.5 - 3.5 mm).

Vaginal smears of red squirrels are similar to those described for rats (Modafferi, 1972), *Rattus norvegicus*, (Long and Evans, 1922) and mice, *Mus musculus* except that in the squirrel, cornified epithelial cells are present in small numbers in diestral and proestral smears; there are also very few leucocytes in post-estral and diestral smears (Modafferi, 1972).

Using the criteria established by Modafferi (1972), three stages of reproduction were recognized from vaginal smears: diestrus, proestrus, and estrus. The properties of the smears that determined the reproductive stage of the female were:

1) percentages of leucocytes, nucleated epithelial cells and cornified epithelial cells,
2) relative number of cells in the smear, and
3) the relative size of nucleated epithelial cells.

Smears were taken by inserting a moist cotton swab into the squirrel's vagina. The swab was dabbed onto a microscope slide and a drop of dilute methylene blue stain was placed on the deposit of vaginal tissue. The number of cells of each type were counted in three microscope fields (100 X).

The animal was recorded as being in proestrus when vulva width was greater than 3.8 mm and the relative abundance of nucleated epithelial cells was scored as "moderate" or "abundant". Proestrus smears also contained more than 40% large nucleated epithelial cells and lacked clumping (cells adhering to other cells in groups). As the female approached estrus, some nucleated epithelial cells were noticably larger than others and all cells showed less clumping. (Thus, it was feasible to estimate the percentage of large versus small nucleated epithelial cells.)

Estrus was usually associated with vulva widths greater than 4.5 mm. An estrus vaginal smear contained only cornified epithelial cells, numbering in the hundreds in the microscope field.

The date of estrus was determined by vaginal smear characteristics, vulva swelling and the animal's behavior. The exact date of ovulation was difficult to detect because vaginal smears with abundant cornified epithelial cells could be obtained for as long as a 5 day period in both captive and wild females. Ovulation was considered to have occurred when a female's vulva changed from being red, turgid and of maximum width to being pale, flaccid and narrow (Dolbeer, 1973). In combination with observations of mating chases and copulations, vaginal smears and vulva swelling could be used to pinpoint the date of ovulation to within two days in wild squirrels. In wild squirrels, parturition was determined either by noting the date when weight loss and onset of lactation occurred, or by backdating from growth rate data collected on captive squirrels.

2.4 Laboratory experiments and analyses

2.4.1 Captive squirrel populations

All squirrels used in the laboratory experiments were trapped in conifer forests near Edmonton and were brought into captivity and care of the Bioscience Animal Services at the University of Alberta, in Edmonton, Alberta. Groups of captive squirrels were established in January 1988, September, 1988 and October, 1989. During most laboratory experiments, squirrels were kept in large rooms with nest boxes, rocks, pine and spruce logs for climbing, and *ad lib* water and food (rat chow, sunflower seeds and jack pine seeds). The rooms were about 5x5x3 m and were kept at 20-25° C. Prior to experiments on photoperiodicity, or during feeding trials with potential phytoestrogens, squirrels were housed in separate cages with a nest box.

Reproduction had not been successful in other studies of captive red squirrels (Smith, 1968; Modafferi, 1972). J. Mahrt (pers. comm.) had difficulty maintaining red squirrels in captivity. Because experiments concerned with light as a proximate cue of estrus were contingent on the ability of red squirrels to cycle in captivity, and on my ability to recognize proestrus and estrus by vaginal smears, a feasibility study was done during January-July 1988. Results indicated that female squirrels had detectable phases of proestrus and estrus in captivity. They also produced litters successfully in the large indoor habitat rooms. The methods and results of the feasibility study are given in Appendix I. They form the basis for other experiments in the laboratory, and they may be useful for captive breeding of endangered squirrels (e.g. Mt. Graham red squirrels).

2.4.2 Food analysis

A preliminary study of foods eaten by wild squirrels prior to estrus was made. Caloric, protein and lipid content of jackpine seeds (Appendix II) and dried mushrooms were quantified using Weende's method of bomb calorimetry, Goldfish lipid extraction (Christie, 1982) and Kjeldahl protein analysis (AOAC, 1975).

2.5 Literature Cited

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior* 49: 227-267.

AOAC. 1975. *Official methods of analysis of the Association of Official Analytical Chemists*. 12th ed., Assoc. Off. Anal. Chem., Washington, D. C.

Christie, W. W. 1982. *Lipid Analysis*. Pergamon Press Canada, Ltd. Toronto, Ont.

Dolbeer, R. A. 1973. Reproduction in the red squirrel (*Tamiasciurus hudsonicus*) in Colorado. *J. Mamm.* 54(2): 536-540.

Gebhardt, S. E. and R. H. Mathews. 1981. Nutritive Value of Foods. *Home and Garden Bulletin* No. 72. USDA. U. S. Government Printing Office.

Gurnell, J. 1987. *The Natural History of Squirrels*. Christopher Helm, Bromley, Kent.

Klenner, W. 1990. *The Effect of Food Abundance and Territorial Behaviour on Population Dynamics of the Red Squirrel*. Ph.D. thesis. University of British Columbia, Vancouver, B. C.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Long, J. A. and H. M. Evans. 1922. The oestrous cycle in the rat and its associated phenomena. *Memoirs Univ. Calif.* 6: 1-148.

Maynard, L. A. and J. K. Loosli. 1969. *Animal Nutrition*. 6th ed. McGraw-Hill book Co. New York.

Millar, J. S. 1968. *The Reproductive Biology of the Western Red Squirrel*. Ph. D. thesis. University of British Columbia. Vancouver, B. C.

Modafferi, M. M. 1972. *Aspects of Reproductive Biology of the Red Squirrel (Tamiasciurus hudsonicus)*. M. S. thesis. University of Alaska. Fairbanks, Alaska.

Pond, C. M. 1984. Physiological and ecological importance of energy storage in the evolution of lactation: evidence for a common pattern of anatomical organization of adipose tissue in mammals. *Symp. Zool. Soc. Lond.* 51: 1-32.

Price, K., K. Broughton, S. Boutin and A. R. E. Sinclair. 1986. Territory size and ownership in red squirrels: response to removals. *Can. J. Zool.* 64: 1144-1147.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels Tamiasciurus. *Ecol. Monogr.* 38(1): 31-63.

Sullivan, T. P. 1990. Responses of red squirrel (*Tamiasciurus hudsonicus*) populations to supplemental food. *J. Mamm.* 71: 579-590.

Zarrow, M. X., J. M. Yochim, and J. L. McCarthy. 1964. *Experimental Endocrinology: a Source Book of Basic Techniques*. Academic Press. New York.

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Chapter 3

Effects of Photoperiod on the Onset of Reproductive Readiness in Red Squirrels: Proestrus & Testicular Recrudescence

3.1 Introduction

Breeding seasons in animals are presumed to be established by natural selection (Baker, 1938; Negus and Berger, 1987). However, for many species the proximate factors that prevent continuous breeding are poorly understood (Clarke, 1981). Correlation between the timing of reproduction and the hours of light per day suggests that many temperate zone mammals are either inhibited, or stimulated by increasing day lengths (decreasing night lengths).

The proximate cues of reproductive readiness are not known for red squirrels (*Tamiasciurus hudsonicus*) (Lair, 1985). As squirrels breed in the spring and summer when day lengths are relatively long, photoperiod may be an important environmental factor in cueing or permitting the gonads to produce gametes. Three laboratory experiments were done to determine whether the photo-environment plays any role in structuring the timing of reproduction in this species.

Day length is an environmental factor to which numerous mammals and birds are sensitve and that some use to time reproduction (Bronson, 1989). Unlike temperature, humidity and food availability, photoperiod is a reliable, "noise-free" annual predictor (Turek and Campbell, 1979). In many species, spermatogenesis in males and onset of reproductive readiness in females are cued and maintained by day length (Clarke, 1981; Bronson, 1989). Many species of *Microtus*, for example, are referred to as "long day" breeders, because they reproduce when day length exceeds some maximum value (usually more than 12.5 h). In contrast, many ungulates (e.g., sheep) are "short day" breeders, having their reproductive systems stimulated or maintained by day lengths of less than 12.5h (Elliot, 1976; Sadleir, 1969). The physiology of photoperiodic control of reproduction in rodents has been reviewed by Karsch (1981), and extensively documented in the Syrian hamster (*Mesocricetus auratus*) and in sheep (reviewed in Bronson, 1989).

For many mammals, the central nervous system, the anterior pituitary gland, the gonads and their target tissues have alternating breeding and non-breeding states. The internal clocks of mammals that coordinate circadian and circannual cycles of physiology, behavior and biochemistry appear to rely on intricate interactions among light, neural input from the retina to the suprachiasmatic nucleus (SCN) and endocrine messengers in the hypothalamus and anterior pituitary (Ralph et al., 1990; Ralph & Menaker, 1988; Miller, 1989).

As reviewed in Binkley (1988), photoperiodic regulation of reproduction in mammals is currently thought to involve complex exchanges between neural and endocrine systems in response to light. First, light stimulates neural receptors on the retina, causing nerve impulses to travel along the retinohypothalamic tract to the SCN in the hypothalamus. These nerve impulses continue through the paraventricular nucleus, then by way of the sympathetic nervous system to the superior cervical ganglia, and finally to the pineal gland (Binkley, 1988). In the pineal, the nerve impulses are transduced to a biochemical signal manifested as the duration of nocturnal melatonin.

Daily and seasonal levels of melatonin secretion depend on the length of daily darkness. An endogenous rhythm entrained by light-dark cycles, or a reproductive physiology that is cued by the photo-environment (see review by Binkley, 1988) may evolve in response to the changes in melatonin. The exact physiological mechanisms by which melatonin influences gonadotropin secretion are unknown, but its main effect is probably to modulate the secretion of gonadotropin-releasing and inhibiting peptides (Bronson, 1989).

The relationship between gonadal cycles and annual environmental change provide clues for what might play a role in timing the breeding of a given species. Tight coupling between a photoperiodic change and a reproductive event is accepted as evidence for day length acting as a proximate regulator (Roberts et al., 1985). On the other hand, abrupt annual alterations in reproductive physiology do occur in the absence of environmental cues. For example, Kenagy (1980) found endogenous circannual rhythms in body weight, testes' size, and water consumption in golden-mantled ground squirrels (*Spermophilus lateralis*) in the absence of potential day length cues. Kenagy (1981) also reports endogenous cycles of reproductive function in the desert ground squirrel (*Ammospermophilis leucurus*). Thus, it is necessary to conduct experiments in which the photo-environment is manipulated and breeding physiology is monitored, rather than accept correlation as cause and effect. With controlled day length experiments, one may determine whether breeding readiness is initiated, prevented or entrained by day length.

North American tree squirrels show a loose coupling between reproductive condition and natural day lengths. All holarctic tree squirrels appear to be long day breeders with modal seasonal onsets of proestrus and estrus occurring after winter solstice (January-May) (Gurnell, 1987). In northern temperate regions (above 40°N), the non-reproductive period (late June to December) is correlated with shortening days (from 18 to six hours of light per day). Onset of breeding condition in the temperate zone is correlated with increasing day lengths (10-14 h).

In red squirrels, proestrus is only observed when day lengths are becoming longer (December-February). Thus, increasing day length is a potential proximate cue for breeding readiness in female red squirrels. However, the dates of ovulation in a given year can vary by as much as 3 months in the same red squirrel population (Rusch & Reeder, 1978), indicating that proximate cues other than, or in addition to photoperiod are required to trigger estrus. Still, day lengths above 10 h may be required for ovulation. By controlling other potential proximate cues, especially food availability and temperature, the hypothesis that long days and/or increasing day lengths are required for proestrus and estrus can be tested. If increasing photoperiod is required to cue proestrus and trigger ovulation, female squirrels housed under short days should fail to become reproductively ready and fail to ovulate. In contrast, females kept under long day photoperiods (16 hours) should exhibit normal or even early proestrus and estrous cycles.

Male squirrels show testicular recrudescence before day length begins to increase suggesting that they have an endogenous circannual rhythm. The male reproductive cycle may depend on the presence of short days (long nights with high levels of melatonin) to which the males become refractory (Zucker et al., 1980). To test this possibility, male squirrels were exposed to continual long day conditions when they should have experienced short days. If short day photoperiods are required to cue recrudescence in males, then squirrels housed in continual long day photoperiods should fail to recrudesce.

3.2 Methods

3.2.1 Experiment 1: Male reproductive response to long and short day photoperiods.

This preliminary trial tested for differences in mean date of testicular recrudescence in male red squirrels kept under continual short and long day conditions. Ten male red squirrels were brought into captivity in October of 1988. Half of the animals were caged in a room that had 16 hours of light per day. The other five were caged in a room with eight hours of day light. Every three days the squirrels were weighed and their testes position (inguinal or scrotal) was recorded. Once the testes had started to enlarge and could be located in the scrotum, external measurements of the length and width of the squirrel's left testis were made using calipers. Developed testes that were inguinal were pushed down into the scrotum for measuring. The Julian date at which each male attained testes of 15 mm or longer (the size correlated with viable sperm (Millar, 1968, 1970; Layne, 1954; this study)) was recorded as the male's recrudescence date. The experiment was maintained until July 15, 1989. Laboratory results were compared with recrudescence dates recorded in the field during the same year.

3.2.2 Experiment 2: Female reproductive response to long and short day photoperiods.

Fourteen female red squirrels were brought into captivity in November of 1989. Half the group was housed in a large room (8x6x3m) with eight hours of day light. The other seven females lived in a similar room in which day length was increased from eight to 16 hours as detailed in Table III-1. Day length was constant at 16 hours per day from January 15 to June 1, when the experiment ended.

Each week the squirrels were weighed, vulva widths were measured and vulva textures were noted as flaccid, partially swollen, or completely turgid (Dolbeer, 1973). A vaginal smear was collected from each female to determine reproductive state (Modafferi, 1972). Timing of proestrus and estrus, defined by the smears and vulva condition, were compared between the two groups. A male squirrel was housed with the females after they were in estrus as part of a captive breeding study.

The following predictions were made:

1. Females in the short day room will not exhibit proestrus vulva-swelling or estrous cycles.
2. Females in the long day room will come into proestrus when day length is longer than 10 h.
3. Females in long day conditions will cycle before wild females given *ad lib* food (see Section 5 for information on food addition experiments).

3.2.3 Experiment 3: Will long day photoperiods in November cause females to exhibit proestrus and ovulation at that time of the year?

A third experiment was conducted to see whether females would cycle in the late fall, (long before the normal breeding season) in response to increasing day lengths. This trial would determine whether day length is used as an immediate and direct cue for timing reproductive readiness. During September of 1988, when days were becoming shorter, ten female squirrels were housed in a large room (as above). Spring was simulated by gradually increasing day length (20 min/wk) in the room from eight to 16 hours as outlined in Table III-1. A male squirrel was housed with the females during the experiment.

Table III-1. Schedule of day length changes in Experiment 2 and 3

Light: Dark h per day	Experiment 2	Experiment 3
10:14	Oct. 30 - Nov. 15	Oct. 1 - Oct. 25
8:16	Nov. 16 - Dec. 15	Oct. 26 - Nov. 5
10:14	Dec. 16 - Jan. 1	Nov. 6 - Nov. 11
14:10	Jan. 2 - Jan. 15	Nov. 12 - Nov. 24
16:8	Jan. 16 - June 1	Nov. 25 - July 10

The occurence and timing of proestrus and estrus in the ten females was compared with the same events in wild female squirrels and in females in Experiment 2. Wild females were subdivided into those that received extra food (food add) and those who did not (controls). All females compared in these groups were multiparous. (See Chapter 5 for details of experiments done with wild squirrels.)

3.3 Results

3.3.1 Do male red squirrels recrudesce differently in long and short day photoperiods?

Mean Julian date of recrudescence in male red squirrels differed among the three environmental conditions (ANOVA, $F_{2,22} = 6.0$, $p < .009$). In the wild, male squirrels recrudesced as early as mid-December and as late as mid-March, a range of about 90 days. Mean Julian date of recrudescence was significantly earlier for males kept in captivity than for those living in the wild (Figure III-1). However, the light regimes in the laboratory had no statistically significant effect on the mean date of recrudescence. Short day males (N=5) had a mean recrudescence date of January 19 while the long day males (N=3) had a mean recrudescence date of December 28 (Figure III-1). Two males housed under the long day photoperiods failed to recrudesce (have testes > 15mm in length), while all other captive and wild males (N=16) became reproductively active (had testes > 15mm) for part of the season.

Mean testes length on February 15 was smaller in males housed under long day conditions than under short day conditions (t=2.6, d.f.=8, p=.032). Males living in 16 h of light per day had a mean testes length of 12.4 ± 9 mm. Males living in short day conditions had a mean testes length of 23 ± 1 mm.

3.3.2 Experiment 2: Do long vs. short day photoperiods cause differences in the timing and occurrence of proestrus and estrus in red squirrels?

Four of the seven females kept under short day conditions exhibited proestrus and estrus, while three failed to come into breeding condition. All seven females housed in the long day photoperiod had vulva swelling and vaginal smears indicative of proestrus, but two failed to exhibit estrus.

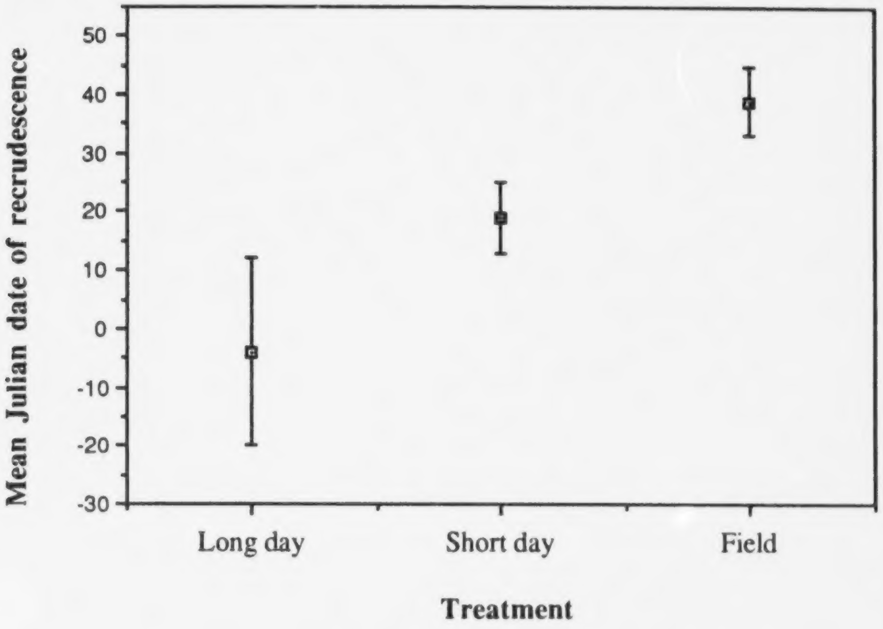

Figure III-1. Mean "Julian" date ± 1 S.E. of recrudescence in male red squirrels. Zero is equal to Dec. 31. Negative numbers result when males recrudesced in December or November. Males (N=3; two failed to attain testes >15mm) housed in long day conditions (16 h light) and short day conditions (N=5; 8 h light) did not have significantly different dates of recrudescence. Laboratory males recrudesced earlier than males in the field (N=16).

The different day lengths affected the timing of reproductive events in the female squirrels (Table III-2). Onset of proestrus was earlier in long day than in the short day treatments (t=2.5, d.f.=9, $p < .017$). The mean date of onset of proestrus was February 17 for females kept under long day photoperiods, but was 5 weeks later (March 25) for females kept under short day photoperiods. Mean date of estrus was also earlier (March 13) in long day females than for the individuals housed in short day conditions (April 11).

(March 13) in long day females than for the individuals housed in short day conditions (April 11).

3.3.3 Results of Experiment 3

Exposure to increasing day lengths in November did not cause female red squirrels to become reproductively ready and ovulate in November. All of the females in this experiment showed proestrus vulva swelling, but only seven out of ten had an estrous cycle. These estrus cycles occurred in the spring but were earlier on average than the estrus dates recorded in the wild and in Experiment 2. Table III-3 summarizes the timing of reproductive events in this experiment. The first record of proestrus or estrus was used to calculate the mean Julian dates of these conditions. Females showing proestrus before January 1 were assigned a Julian date of 0.

Female squirrels exposed to increasing day lengths in November came into proestrus earlier than females in the wild and females in Experiment 2 (Figure III-2). The mean date of proestrus (Julian day 19 ± 23 days) occurred about a month earlier in this experiment than in the long day group in Experiment 2 (ANOVA $F_{4,45}=8.4$, $p=.0001$).

When the results of laboratory and field experiments were combined, there was no significant difference in timing of proestrus between wild controls and females kept in short days. Wild squirrels feeding on *ad lib* sunflower seeds exhibited proestrus at the same time as the females that were exposed to long days after Dec 15 (Figure III-2).

Table III-2. Chronology of reproductive events in red squirrels under two light regimes in Experiment 2 (Jan. 15 - June 1, 1990). The intervals of proestrus and estrus are defined by first and last dates of vaginal smears typical of those reproductive stages. Single dates indicate that diestrus smears were obtained in some weeks.

Squirrel I.D.	Light Regime	Proestrus	Estrus
bR 6260	16L: 8D	Jan 18 - Feb 20	Feb 27- Mar 28
WW 1030	16L: 8D	Jan 27 - Feb 27	Mar 6 - Mar 28
bB 1435	16L: 8D	Feb 1 - Mar 12	Mar 19 - Mar 28
WY 1092	16L: 8D	Feb 27 - Mar 12	Mar 19 - Mar 28
bb 1406	16L: 8D	Feb 27 - Mar 12	Mar 19 - Mar 28
Bb 1043	16L: 8D	Feb 27 & Mar 19	None
bW 1438	16L: 8D	Feb 1, 27; Mar 19	None
GR 1612	8L: 16D	Mar 19 - Mar 28	Apr 9 - May 28
Ob 6231	8L: 16D	Mar 19 - Mar 28	Apr 9 - May 4
WO 7305	8L: 16D	Mar 12 - Mar 19	Apr 9 - May 28
bb 6262	8L: 16D	Apr 9	Apr 19 - May 28
RB 1409	8L: 16D	None	None
bO 6238	8L: 16D	None	None
WB 1131	8L: 16D	None	None

Table III-3. Photoperiod at onset of proestrus and dates of proestrus and estrus (Exp.2)

Squirrel I.D.	Light Regime	Proestrus	Estrus
bY 378	16L: 8D	Jan 1 -Feb 23	None
bG 7405	16L: 8D	Jan 12 - Jan 19	None
G/W 7408	16L: 8D	Dec 1 - Mar 3	Mar 6-18
bW 7830	16L: 8D	Jan 12, Feb 10	Apr 10-29
bR 7841	16L: 8D	Feb 17 - Mar 15	Apr 7
bO 7861	16L: 8D	Jan 14, Feb 21	Mar 6-17
bG 7910	16L: 8D	None	None
bB 7955	16L: 8D	Feb 10 - Mar 1	Mar 3-15
WW 7896	10L: 14D	Nov 8; Jan 2	Feb 27
OW 7365	16L: 8D	Jan 1-Feb 27	Mar 3-Apr 7

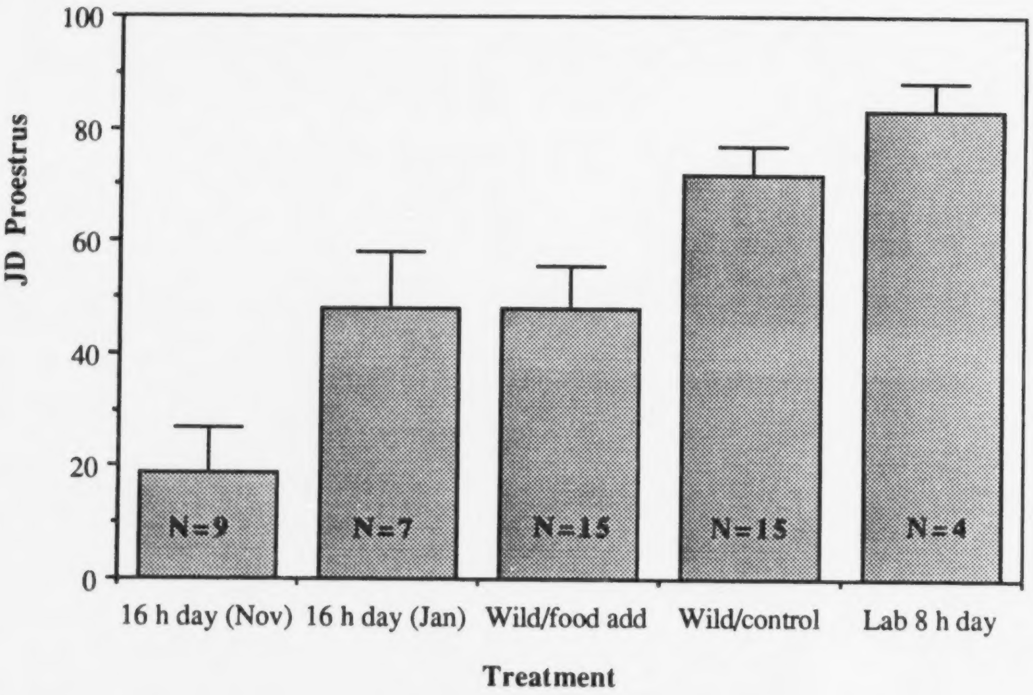

Figure III-2. Mean Julian date + 1 S.E. of the first sign of proestrus vulva swelling and proestrus vaginal smears in red squirrels as a result of different treatments. Julian date 1 = Jan 1. Females showing proestrus before Jan 1 (N=2) were assigned a Julian date of 0 in the calculations.

Day length manipulations and food availability shifted estrus dates in a pattern similar to that noted for proestrus (ANOVA $F_{4,42}$= 17.9, p=.0001). The only difference was that the mean date of estrus for females exposed to long days in November showed no statistical deviation from the mean for females housed under long day photoperiods in Experiment 2 (Figure III-4).

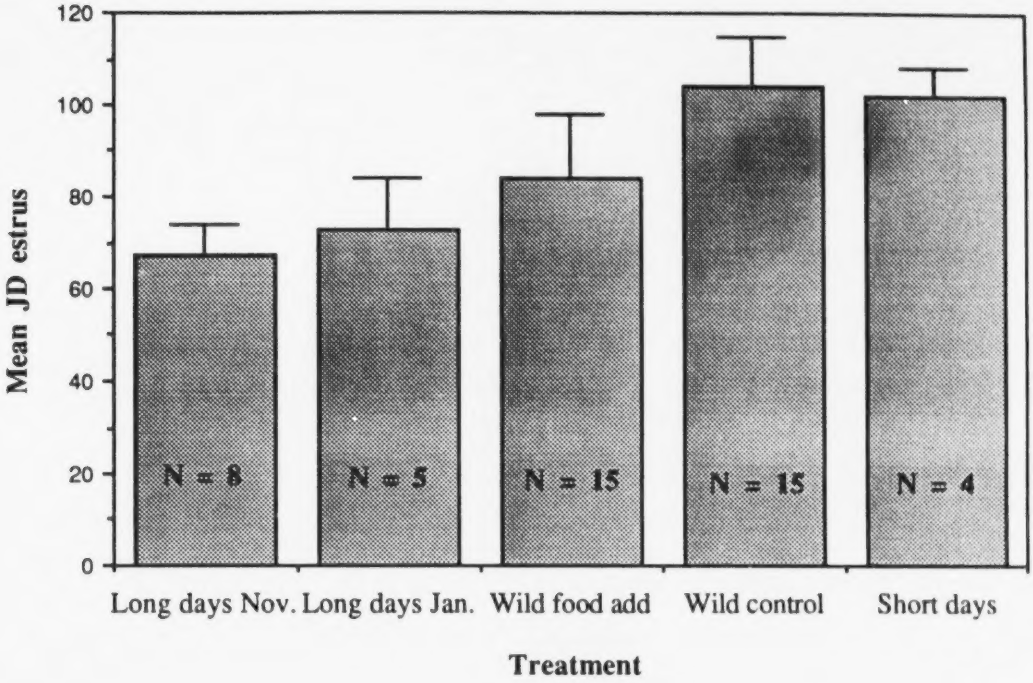

Figure III-3. Mean Julian date + 1 S.E. of the first sign of estrus vaginal smears in red squirrels as a result of different treatments. Julian date 1 = Jan 1.

3.3.4 Other comparisons between captive and wild squirrels

In comparison with the wild squirrels, the captive females had prolonged periods of estrus as indicated by vaginal smearing. Wild squirrels had estrus vaginal smears lasting at most five days. In contrast, squirrels in captivity produced large vaginal amounts of squamous epithelial tissue typical of estrus for two to four weeks. In the field, 100% of the multiparous squirrels given *ad lib* food (N=15) had an estrus, while only 71% of the females in the captive settings (N=24) had an estrous cycle.

Despite *ad lib* food and mild temperatures in the laboratory settings, the proportions of females producing a litter in the lab were smaller than predicted given performance in the wild population ($\chi^2 = 13.2$, d.f.=1, $p < .01$). All the wild multiparous squirrels given *ad lib* food produced litters, while only 2 of the 17 captive females that ovulated conceived and gave birth in captivity.

3.4 Discussion

3.4.1 Preliminary findings in male red squirrels

Laboratory conditions with plentiful food and no thermal challenges probably explain why testes development was ealier in captive males than in wild male squirrels. Food supplementation is known to advance and lengthen the breeding season in many mammals (Boutin, 1990). Under similar photoperiods, wild female squirrels given food supplements have estrus cycles 3 to 4 weeks earlier than females without extra food (Chapter 5). In male hyrax (*Procavia capensis*) testes development was reduced when animals were on a reduced calorie diet (Clarke, 1981).

Males in both short and long day conditions showed normal testes development. Two of the five males kept in long day conditions failed to recrudesce, while wild (N=16) and short day (N= 5) males developed testes of reproductive size. Continual long days (lack of short days) may have retarded seasonal testes development in these squirrels. The two males were adults and were in excellent body condition, so there were no obvious alternative explanations for their failure to recrudesce.

Male red squirrels may vary in responsiveness to photoperiod. Laboratory studies have found that some individual voles of the genus *Microtis* are reproductively active despite long-term maintenance in short day lengths (Nelson, 1987). These animals have been referred to as "photoperiod-nonresponsive morphs." Larger experimental groups and year round monitoring are needed to better elucidate the importance of photic cues in male red squirrels.

3.4.2 Response to photoperiod in female red squirrels

In contrast to the original prediction that short days would prevent reproduction, some of the female squirrels kept in short day conditions became reproductively active and had estrous cycles. The females that became reproductive in short day conditions may have been photoperiod nonresponsive morphs (Nelson, 1987) or they may have been responding to endogenous circannual rhythms (Kenagy, 1980). All the females were multiparous adults, so entrainment of endogenous reproductive cycles in response to day length cues was possible during previous seasons. Given lifespan of the red squirrel, a circannual rythms is more likely than the presence of morphs with different genetic-based responses to day length (Bronson, 1989).

The shifts in proestrus noted in the red squirrel experiments suggest that female red squirrels have a circannual rhythm of reproduction that is entrained by day length. Onset of proestrus was advanced by exposure to long day conditions. The earlier in the year that females experienced long days, the earlier they exhibited proestrus. In contrast, the proestrous and estrous cycles of females housed under short day conditions were later in the season than those of the long day females. Apparently, the lack of long days causes a significant delay in the onset of reproduction. These results suggest that circannual reproductive cycles of red squirrels may be entrained and modified by photoperiod. When other light-sensitive animals experience shifts in the timing of potential photic cues, such as trans-equatorial moves, there is often a gradual change in the timing of reproduction during a re-entrainment phase (Clarke, 1981; Karsch 1981).

Long days did not immediately trigger proestrus in all of the captive squirrels. Most of the females exposed to increasing photoperiods did not show a consistent and quick change from diestrus to proestrus at the same time that natural days became long. Only one of the ten females in Experiment 3 had a swollen vulva and vaginal smears indicative of proestrus close to the time when day length exceeded 10-12 h. Delayed response was also noted in squirrels housed in long day conditions in Experiment 2. Most cases of proestrus were recorded more than a month after the time when day length became longer than 12 h. These results are inconsistent with patterns of proestrus in wild squirrels. In the wild, proestrus typically occurs at the same time that the days elongate from 10 to 14 h: between February 15 and April 15. The lag in response to artificially produced long days further supports the idea that sensitivity to long days depends upon a circannual rhythm.

That red squirrels have endogenous circannual reproductive cycles is not surprising. One would predict the presence of a circannual rhythm in a fairly long-lived seasonal breeder (Bronson, 1989). However empirical evidence of such annual cycles is lacking for most wild mammals.

Could other factors besides light have caused proestrus to coincide with early exposure to long days? Because food and social setting were the same in all the laboratory experiments, they may be eliminated as potential factors. Perhaps a dominant female in each group triggered the others through pheromones and the timing patterns reflect this (Dewsbury, 1982). Even if this were the case, it can be argued that the timing of long day conditions influenced the dominant female in each group. Also, if this were the case, one would expect more synchrony in the onset of proestrus than was found in each experimental group.

Repeating the experiments with juvenile female squirrels would be one way to eliminate the potential effects of developmental entrainment. However, in a preliminary laboratory trial with 6 females housed in 12L:12D, the three juvenile red squirrels failed to show any reproductive activity in captivity, while the three multiparous females came into estrus, ovulated and produced two litters. (see Appendix I).

3.4.3 Why were reproductive events in wild squirrels different from those in the laboratory?

Husbandry effects probably explain low rates of ovulation and conception in the laboratory experiments. Research conducted on grey squirrels (*Sciurus carolinensis*) indicated that "space" was necessary for the females to come into estrus (Wembly & Johnson, 1983). In this study, the average space per red squirrel was reduced from about half a hectare to less than 10 cubic meters. In the wild, each female red squirrel defends a territory and has little physical contact with other squirrels. During these laboratory trials, female squirrels were observed in groups of two to four animals, sharing nest boxes, and even grooming each other.

Although there were no obvious dominance hierarchies in the female squirrels, it is possible that some females inhibited ovulation and pregnancy in others. Pheromones from female rats are known to synchronize ovulation of other rats housed in the same room (McClintock, 1983). In the naked mole rat (*Heterocephalus glaber*) only one female breeds, while the rest of the females in the colony are repressed by some factor (probably pheromonal) associated with the breeding female (Jarvis, 1981).

In conclusion, artificial exposure to long day conditions can advance the timing of proestrus. However, if a photic cue is not available, squirrels are still capable of breeding later in the year. These results suggest that (1) endogenous rhythms of reproductive condition are present in red squirrels (2) these endogenous cycles are entrained by day length, and (3) artificially created short days delay but do not prevent reproduction.

3.5 Literature Cited

Baker, J. R. 1938. The evolution of breeding seasons. In: G. R. de Beer (ed.) *Evolution: Essays on Aspects of Evolutionary Biology*, Oxford University Press, London, 161-177.

Binkley, S. 1988. *The Pineal*. Prentice-Hall. New Jersey.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems and the future. *Can. J. Zool.* 68: 203-220.

Bronson, F. H. 1989. *Mammalian Reproductive Biology*. University of Chicago Press, Chicago.

Bronson, F. H. 1985. Mammalian reproduction: an ecological perspective. *Biol. Reprod.* 32: 1-26.

Clarke, J. R. 1981. Physiological problems of seasonal breeding in eutherian mammals. In: C. A. Finn. (ed.). *Oxford Reviews of Reproductive Biology*. Oxford, Clarendon Press.

Dewsbury, D. A. 1982. Dominance rank, copulatory behavior and differential reproduction. *Quart. Rev. Biol.* 57: 135-60.

Dolbeer, R. A. 1973. Reproduction in the red squirrel (*Tamiasciurus hudsonicus*) in Colorado. *J. Mamm.* 54(2): 536-540.

Gurnell, J. 1987. *The Natural History of Squirrels*. Christopher Helm, London.

Karsch, F. J. and Foster, D. L. 1981. Environmental control of seasonal breeding: a common final mechanism governing seasonal breeding and sexual maturation. In *Environmental factors in mammal reproduction*, D. Gilmore and B. Cook (eds.), 30-53. Macmillan. Hong Kong.

Kenagy, G. J. 1980. Interrelation of endogenous annual rhythms of reproduction and hibernation in the golden-mantled ground squirrel. *J. Comp. Physiol.* 135:333-39.

Kenagy, G. J. 1981. Endogenous annual rhythm of reproductive function in the non-hibernating desert ground-squirrel *Ammospermophilis leucurus*. *J. Comp. Physiol.* 142: 251-58.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

McClintock, M. K. 1983. Pheromonal suppression of the ovarian cycle: enhancement, suppression and synchrony. In: *Pheromones and Reproduction in Mammals*, J. G. Vandenberg (ed.), 113-50. New York: Academic Press.

Millar, J. S. 1970. The breeding season and reproductive cycle of the western red squirrel. *Can. J. Zool.* 48: 471-473.

Millar, J. S. 1968. *The Reproductive Biology of the Western Red Squirrel*. Ph. D. thesis. University of British Columbia. Vancouver, B. C.

Miller, J. A. 1989. Clockwork in the brain. *Biosci.* 39: 75-78.

Modafferi, M. M. 1972. Aspects of Reproductive Biology of the Red Squirrel (*Tamiasciurus hudsonicus*). M.S. thesis. University of Alaska. Fairbanks, Alaska.

Negus, N. C. and P. J. Berger. 1987. Mammalian reproductive physiology: adaptive responses to changing environments. In: *Current Mammalogy*. New York, Plenum Press. 149-173.

Nelson, R. J. 1985. Photoperiodic regulation of reproductive development in male prairie voles: influence of laboratory breeding. *Biol. Reprod.* 33: 418-422.

Nelson, R. J. 1987. Photoperiod-nonresponsive morphs: a possible variable in microtine population-density fluctuations. *Am. Nat.* 130: 350-369.

Ralph, M. R. and M. Menaker. 1988. A mutation of the circadian system in golden hamsters. *Science* 241:1225-27.

Ralph, M. R., R. G. Foster, F. C. Davis and M. Menaker. 1990. Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247: 975-78.

Roberts, A. C., N. D. Martensz, M. H. Hastings and J. Herbert. 1985. Changes in photoperiod alter the daily rhythms of pineal melatonin content and hypothalmic beta endorphin content and the lutenizing hormone response to naloxone in the male Syrian hamster. *Endocrinology* 117: 141-48.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

Sadlier, R. M. F. S. 1969. *The Ecology of Reproduction in Wild and Domestic Mammals*. Methuen. London.

Turek, F. W. and C. S. Campbell. 1979. Photoperiodic regulation of neuroendocrine-gonadal activity. *Biol. Reprod.* 20:32-50.

Webley, G. E. and E. Johnson. 1983. Reproductive physiology of the grey squirrel *Sciurus carolinensis*. *Mammal Rev.* 13: 149-54.

Zucker, I., P. G. Johnston and D. Frost. 1980. Comparative, physiological and biochronometric analysis of rodent seasonal reproductive cycles. *Prog. Reprod. Biol.* 5:102-33.

Chapter 4

Variation in Body Weight and Body Fat in Female Red Squirrels

4.1 Introduction

Body weight is one of the most frequently measured variables in organismal and population-level studies of vertebrates. It is typically the dependent variable in intraspecific studies of growth and aging, effects of dietary change, or effects of altered environmental conditions (Eisenberg, 1981). In several species, body weight has been a good predictor of the amount of body fat that an individual animal maintains (Albon *et al.*, 1986), and has been positively correlated with rank, productivity, and reproductive success (Albon *et al.*, 1986; Clutton-Brock, 1988; Wauters & Dhondt, 1989b).

Four questions were explored in this study focusing on variation in body weights of female red squirrels:

1. What are the annual patterns of weight change in red squirrels?
2. To what extent do age and reproductive experience correlate with weight and fat stores in red squirrels?
3. Is weight a good index of body fat stores in live squirrels?

In contrast to most studies of body condition, in which fairly large numbers of animals are killed, this study employed a non-destructive fat analyser to determine body fat levels in living wild squirrels.

4.2 Methods

4.2.1 Body fat analysis in red squirrels: laboratory and field techniques

A portable fat analyzer constructed by EM-SCAN, Inc (P. O. Box 10, Auburn, IL 62615) was used to measure the body fat in live squirrels. Originally designed for use on hamburger, the ME-100 fat analyzer measures total body electrical conductivity (Presta *et al.*, 1983; Walsberg,1988). When a live animal is placed into the EM-100's measurement chamber, the electromagnetic inductance around the chamber is altered. Electrical conductivity of lean tissue is greater (by approximately 95%) than that of fat because of the much higher electrolyte content of lean tissue (Presta *et al.*, 1983). Thus, the signal output produced by the ME-100 fat analyser is mainly dependent on the amount of lean tissue in the sample (Walsberg, 1988). An internal microprocessor generates a set of numbers that permit the calculation of a variable (called the EMME number), a complex function of the size, shape and conductivity of the material in the measurement chamber.

To quantify body fat using total body electrical conductivity (TOBEC), a calibration curve relating EMME numbers to lean tissue for a given species is required. Twenty-three red squirrels (10 adult males, 10 adult females and 3 juvenile females) were used to generate the calibration curve. First, EMME numbers were determined by placing each animal in the ME-100 analyzer. A mean EMME value was calculated from six measurements. The variation caused by the non-cylindrical shape of the squirrel was averaged by centering the animal in the cylinder three times with the head pointed inwards, and three times with the head pointing out.

After making the live measurements, the squirrels were killed using carbon dioxide gas. The carcasses were autoclaved at 5 p.s.i. at 120°C for 12 hours, homogenized with water in a blender, and freeze-dried. Each freeze-dried carcass was crushed into a homogeneous powder. Neutral lipids (Allen, 1976) were extracted in Goldfish extractors from triplicate samples of each powdered carcass using petroleum ether as the solvent (Christie 1982). Fat to lean ratios of the samples were used to estimate the fat and lean masses of the whole carcasses. Values for lean body mass were regressed on the mean EMME numbers for the sample of 23 squirrels.

The regression was used in the field to extrapolate lean body mass (LBM) of live squirrels. Subtraction of LBM from an animal's total body weight resulted in a TOBEC estimate of fat for the wild squirrels.

To determine the relationship between triglycerides and percentage of body fat, seven fat samples obtained from squirrel carcasses were run as duplicates through an Iatroscan process. This flame-ionization process separates the lipids: cholesterol, triglycerides, and free fatty acids, and quantifies the relative abundance of each type (Christie, 1982).

4.2.2 Field studies of body weights of female squirrels

Field studies on red squirrels were conducted over a three year period, 1988-90, near Fort Assiniboine, Alberta, at the Holmes Crossing area of the Athabasca Sandhills (54°17' N, 114°45'W). The site is dominated by an uneven-aged stand of jack pine (*Pinus banksiana*), the seeds of which form the main diet of the red squirrels in the study population. The climate at the site is extremely seasonal, with below freezing temperatures prevailing from December through mid-March (Section 2.2).

Red squirrels were trapped (Price *et al.*, 1986) at their middens, the site of each squirrel's main food cache and below-ground nests. Tomahawk live-traps baited with peanut butter were set on top of each midden, and were left open for no longer than two hours between checking for captures. During the fall, winter and spring, ear-tagged female squirrels were recaptured at least twice per month and, weight (grams), frame size (zygomatic width in mm), and reproductive condition were assessed. Zygomatic width was used as an index of frame size because it is strongly correlated with other morphometric traits of squirrels, such as hind-foot length, cranial width, total body length and tail length (Appendix III).

Female squirrels were classed by their reproductive experience or by age when year of birth was known. Reproductive classes were distinguished by nipple size and pigmentation (Layne, 1954). Females that had not attained puberty were readily identified by pink nipples, less than 1.0 mm long. Nulliparous, cycling females had pigmented (black-brown) nipples that were less than 1.5 mm in length. Multiparous females had noticeably elongated nipples (> 2.0 mm) that were usually pigmented. Multiparous females were all at least two years old, and some were as old as six years at the end of the study. Juvenile squirrels were less than a year old. Squirrels producing a litter for the first time are referred to as primiparous females.

Squirrel weights obtained during trapping sessions were analyzed for differences due to season, reproductive class, frame size, age and body fat. Growth was calculated using change in zygomatic width as a measure.

4.3 Results

4.3.1 Calibrations and comparisons of body fat stores

The relationship between lean body mass (LBM) and the mean EMME numbers generated by the fat analyzer had moderate predictive value ($r^2= 0.5$, $p=.0005$). The equation for the line predicted by the data was: $Y= 0.2x + 111.1$, where Y = LBM and x=EMME number (Figure IV-1). Juvenile squirrels account for two of the lowest masses in Figure IV-1, suggesting that they may be small and lean enough to require a different equation from adults. Because one adult female was of a size similar to the juveniles, the data for juveniles were kept in the regression.

TOBEC estimates of body fat were calculated using the following equation:

TOBEC fat (g)= Total carcass weight (g) - 0.2 EMME + 111.1

The linear relationship between "real" fat extracted from squirrel carcasses and body fat estimated using TOBEC was strong (r^2=0.8, p=.0001, Figure IV-2), but the TOBEC system over-estimated values of extractable fat by a factor of 1.3.

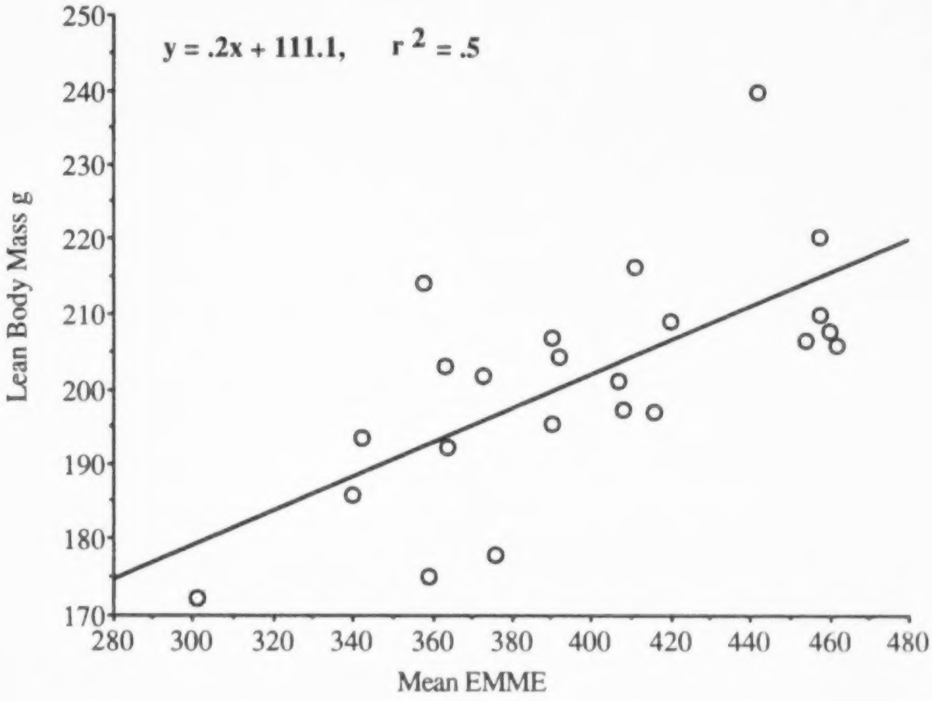

Figure IV-1. Linear relationship between EMME numbers determined by measuring total body electrical conductivity (TOBEC) and lean body mass (LBM) of 23 red squirrels.

Weight alone was as good a predictor of body fat in the sample of 23 red squirrels (r^2= 0.8, p= 0.0001) as the TOBEC estimates. Figure IV-3 shows the regression of the amount of carcass fat determined by the Goldfish extractions on body weight. When included as a covariate in a multiple regression, frame size (zygomatic width) did not improve the fit of the relationship. The partial F value for zygomatic width was only 0.02.

Triglyceride levels were positively correlated with an increase in total carcass lipids: $y = 80.1 + 0.963x$ ($r = 0.95$, $p < 0.0001$, $N=7$); where y is the percentage of triglycerides and x equals the percentage of fat extracted.

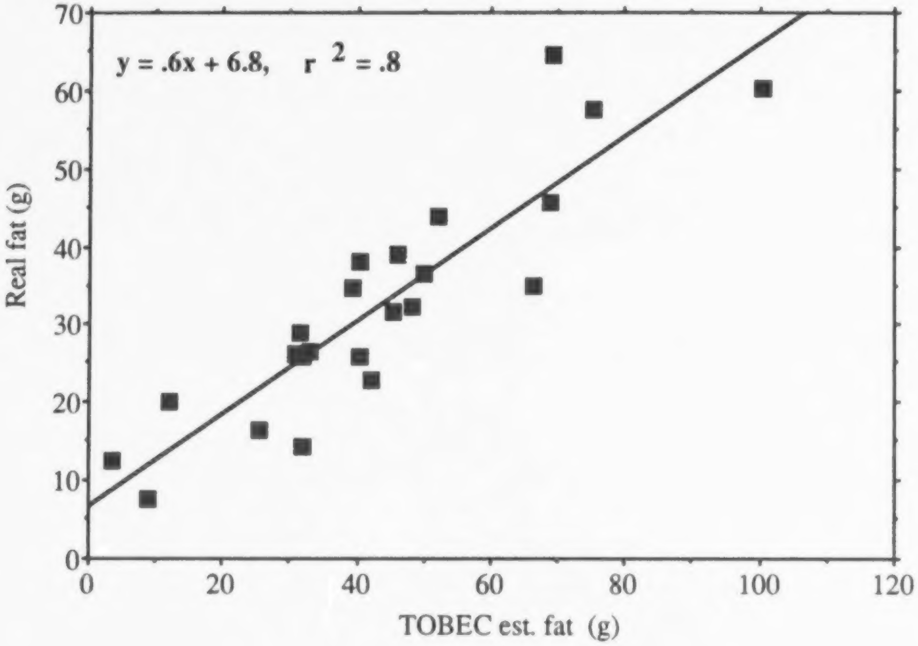

Figure IV-2. The linear relationship between the TOBEC estimate of fat (total weight minus the lean body mass estimated by the ME-100 fat analyzer) and the amount of fat determined by laboratory fat extractions ("real" fat).

4.3.2 Seasonal, age and reproductive class effects on weight

Weights of female red squirrels were affected by age, reproductive class, and time of the year. Seasonal means of body weights in the three classes of females are shown in Figure IV-4. Juvenile females were the lightest, while multiparous females were the heaviest (2-factor repeated measures ANOVA, $F_{2,57}$=48.1, p= 0.0001). Peak weights during the non-breeding seasons occurred in late fall (October-December), while the lowest weights were recorded in late January through early March (2-factor repeated measures ANOVA by month, $F_{4,57}$=50, p= 0.0001). On average, adult females increased in body weight during February and March before breeding, while juveniles continued to decline in average mass at this time of the year. High weights in April reflect pregnancies in some females.

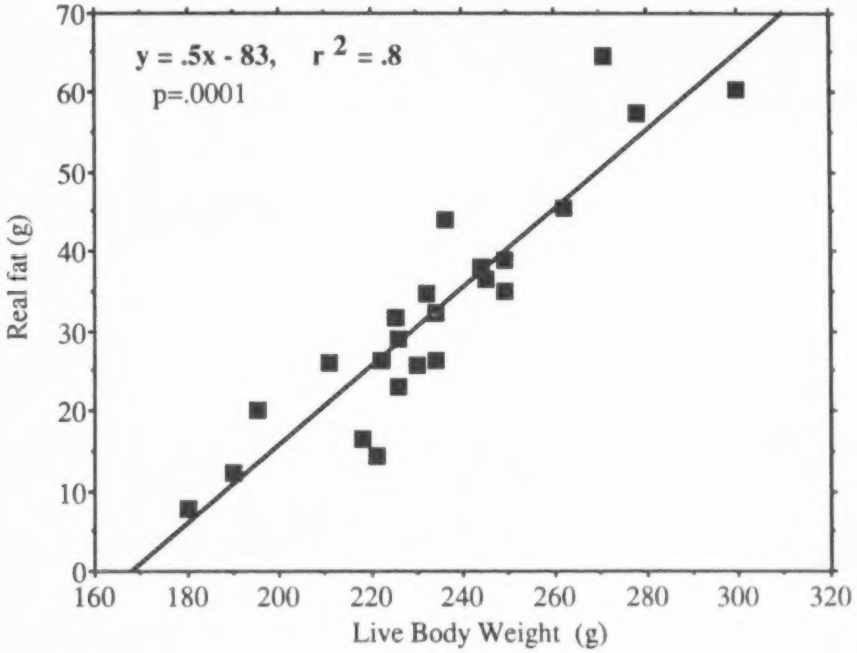

Figure IV-3. Linear relationship between body weight and body fat in red squirrels.

Weights were measured for a smaller number of females during September 1988 to April of 1989. Figure IV-5 illustrates the variation in these weights due to age and season.

The "winter" mean was composed of the lowest weight recorded for each female between mid-December and March 15th. The "fall" and "spring" weights were an average of 5-8 measurements made on each squirrel. Fall weights were taken from September to mid-December, while spring weights were taken from mid-March to early May and include pregnancies. Summer weight patterns were not monitored during this study.

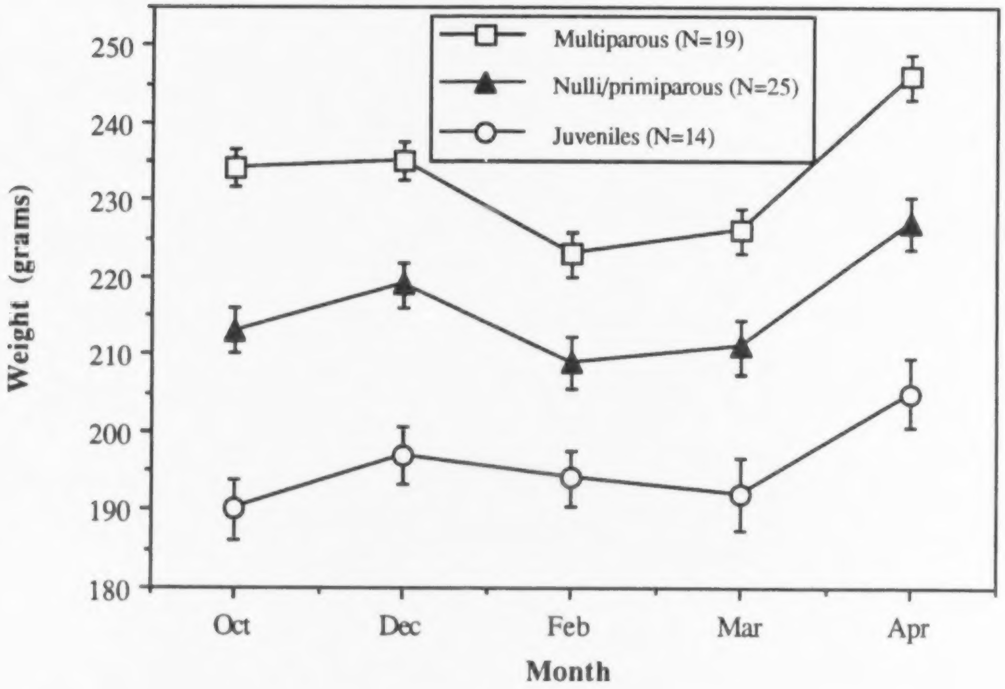

Figure IV-4. Plots of mean weight ± 1 S.E. of female red squirrels (1989-90). The weight of a female squirrel depends upon her age, reproductive status, and the time of year. High April means reflect pregnancies.

On average, nulliparous females had lower weights than multiparous females throughout the entire non-breeding season. These differences were highest in the fall when breeding females weighed an average of 25 grams more than nulliparous females. The lowest average weights exhibited by females during the winter months showed a smaller difference, varying by only 15 grams (Figure IV-5).

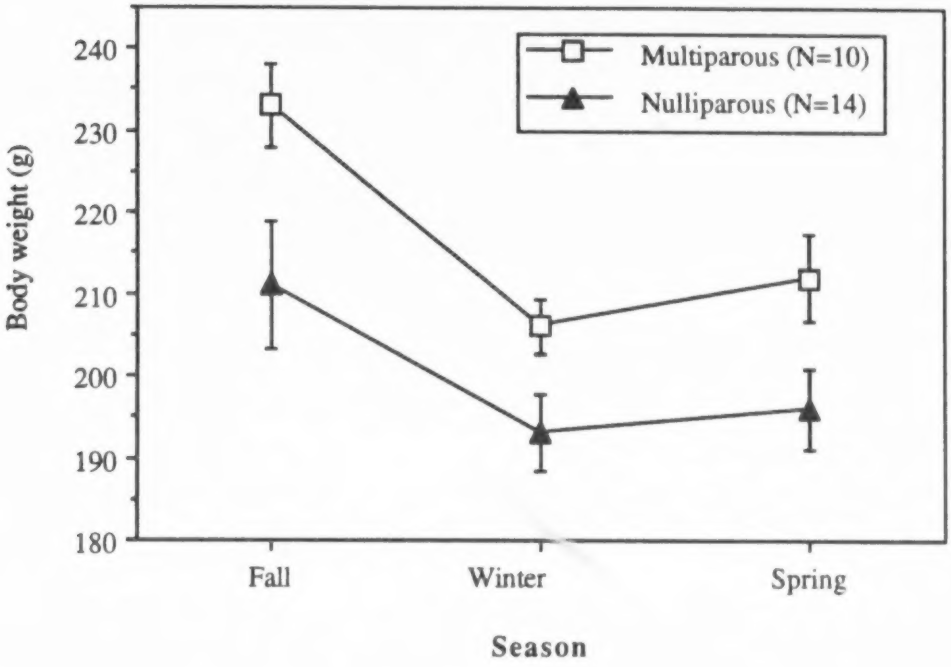

Figure IV-5. Plots of mean weight (grams) ± 1 S. E. of female red squirrels (1988-89). Nulliparous females were lighter weight than multiparous females in all non-breeding seasons. Lowest winter weights were recorded during January, February and early March. Sample size (N) refers to the number of different squirrels measured.

Duplicate weight measurements of the same squirrel, made on the same day, varied by as much as 8 grams. The difference probably reflects variable stomach content, hydration, time in the trap, and error in reading spring balances.

4.3.3 Frame size and growth effects

Measurements made in October 1989 on 108 wild female squirrels show that weight and zygomatic width is strongly correlated (r=0.8, p< 0.0005; Figure IV-6).

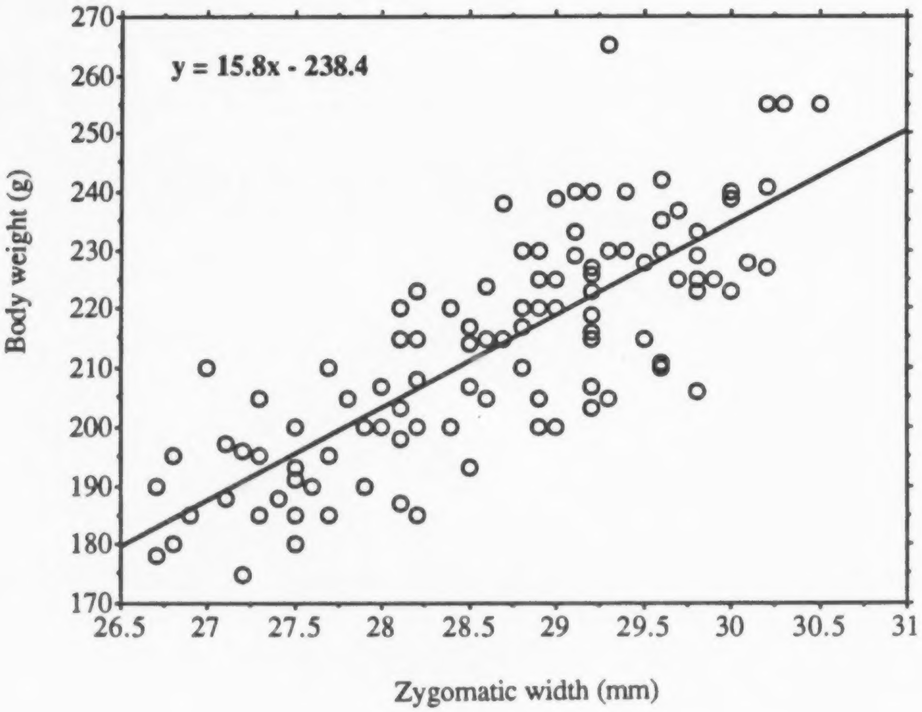

Figure IV-6. Relationship between body weight and zygomatic width in female red squirrels. Data from 108 females measured in September, 1989.

Juveniles and nulliparous females showed significant growth in zygomatic width over a six month period (October to April of 1989-90), while multiparous adult females did not. In a two-factor repeated measures ANOVA of zygomatic widths, both age/reproductive class and the repeated measure explained significant amounts of the variance in zygomatic widths ($F_{2,62}$=22.5 P=.0001; $F_{2,62}$=69.1; P=0.0001 respectively). The mean growth of 0.8 mm in the zygomatic arches of juveniles was large enough to cause an interaction effect (F=24.9, P=0.0001). Multiparous adult squirrels showed no growth, while nulliparous adults showed a small mean growth increment of 0.3 mm over the six months (Figure IV-7).

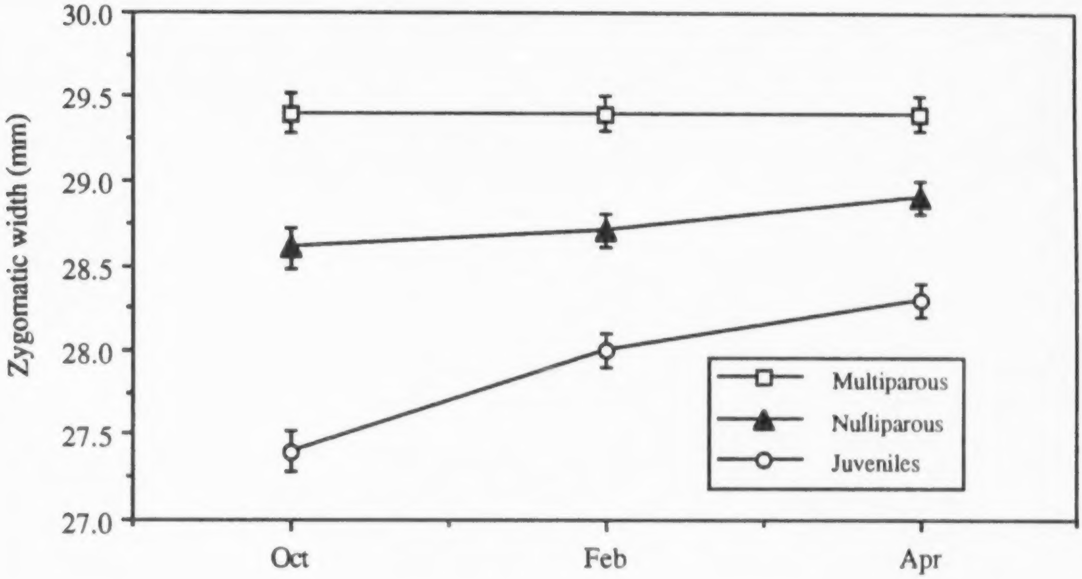

Figure IV-7. Over-winter (October -April) change in mean zygomatic widths of female red squirrels. Multiparous squirrels (N=19) showed no growth while nulliparous (N=27) and juvenile females (N=16) show slight or substantial growth.

These results suggest that weight changes in adult multiparous females are mainly due to change in fat stores, while those observed in juveniles and nulliparous adult females, result from a combination of body growth (changing frame size) and changing fat stores.

4.3.4 Do changes in weight during spring represent changing fat levels?

To determine whether fluctuations in weight during the spring were due to changing fat stores or changes in lean mass, TOBEC measurements were made on 17 adult female squirrels in February and again in March. Change in fat as measured by the ME-100 fat analyzer accounted for 50% of the change in weight in this sample (Figure IV-8). In the same analysis, change in lean body mass accounted for about 28% of the variance in weight (r^2=0.278, p=0.03).

There was no difference in mean frame size of the females during the study period, so the changes were not the result of growth *per se*. Estimates of actual body fat differed for the two reproductive classes of females in the sample. Multiparous squirrels had an estimated mean of nine grams of body fat (4-5% body weight) in February, while the mean estimated for nulliparous females was less than 1 gram (t=2.8, d.f.=15, $P< 0.05$).

4.3.5 Did age affect relative amounts of fat before the breeding season?

Both nulliparous adults and juvenile female squirrels had attained more than 90% of their full adult mass before the breeding season in March. With a mean lean mass of 180 ± 6.5 grams, juveniles (N=22) averaged 92% of the mean lean mass calculated for multiparous females (N=29). Nulliparous adult females (N=33) averaged 10 grams heavier in lean mass than the juveniles, or 97% of the mean calculated for multiparous adult squirrels (Figure IV-9).

During March, both the juveniles and the nulliparous adult females had lower estimates of absolute body fat than multiparous female squirrels (ANOVA, $F_{2,83}$=34.3, p=0.001). However, this was not simply a consequence of body size. When scaled for body size, using the relationships of lean mass described above, juveniles had on average half as much fat as multiparous females. To be proportionally the same as multiparous females, juveniles should have had a mean of 27 grams of stored fat (92% of the estimated mean in multiparous females), but they averaged only 14 grams. Nulliparous females had 80% of the fat stores predicted for their body size, a mean of 23 grams of fat. As an averaged whole percentage of body weight, fat levels measured in mid-March were 13% in multiparous females, 11% in nulliparous females and only 7.2% in juveniles.

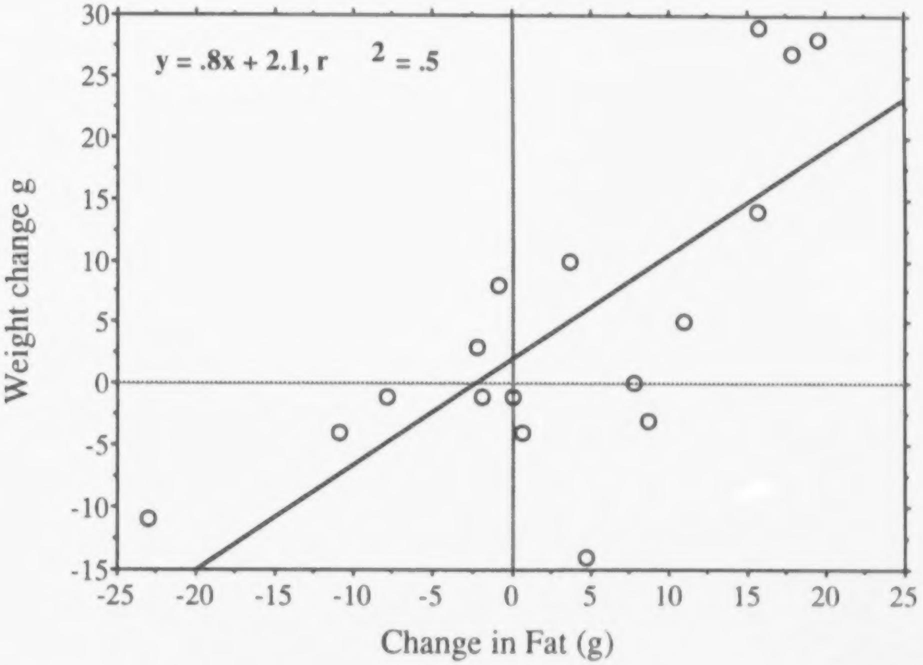

Figure IV-8. Linear relationship between change in body fat and change in body weight of female red squirrels. Changing fat stores estimated by TOBEC explained 50% of the variation in spring weights of red squirrel females.

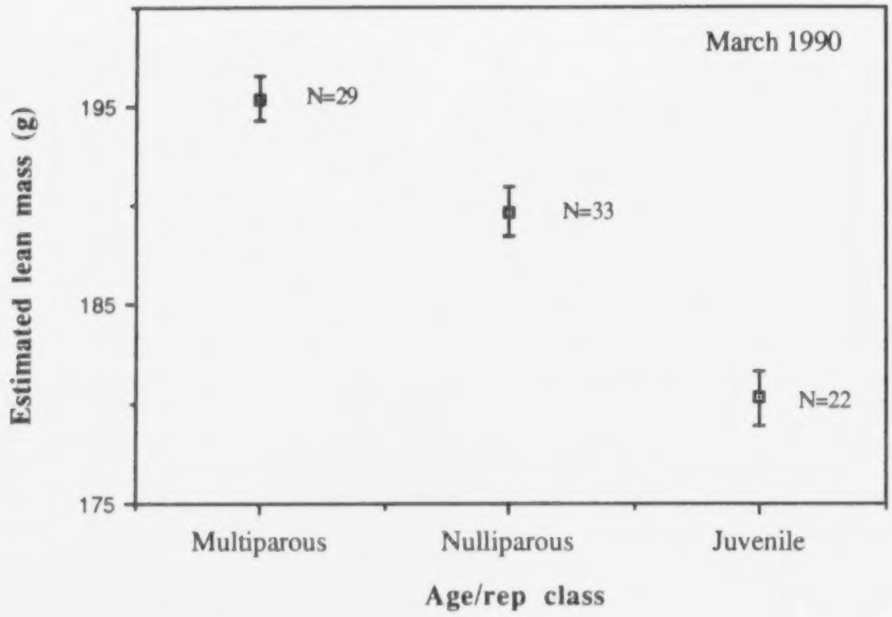

Figure IV-9. Estimates of the mean lean mass ± 1 standard error in red squirrel females during March.

4.4 Discussion

4.4.1 ME-100 fat analyser and calibrations

The linear regression of EMME numbers on lean body mass had more unexplained variation than calibration curves published by other authors. Kenagy (1989) established calibration curves for golden-mantled ground squirrels. In two separate trials with sample sizes similar to those in this study, Kenagy (pers. comm.) attained strong linear relationships between lean mass and EMME numbers (significant r^2 values above 0.9). Walsberg (1988) had similar results to Kenagy when he used an ME-100 fat analyser on a large collection of reptiles, birds and mammals. My results were similar to Presta *et al.*

(1983) measurements of body composition in 19 humans. They found that EMME numbers had a moderate linear relationship with lean body mass (r^2=0.48).

Because the results of the Goldfish fat extractions showed little variation, I concluded that most of the variation in my calibration curve came from the condition and position of the squirrels in the fat analyser rather than from laboratory procedures. Hydration and temperature of animal tissue are known to affect EMME values (Walsberg, 1988). Rectal temperatures of squirrels in the study varied from 39 to 41°C, and hydration of the live squirrels may have varied as well, although the animals had access to an unlimited supply of water. Metal eartags had a negligible effect on the readings.

EMME numbers reflect the amount of lean tissue in a sample, but they vary with the size and shape of a sample. Although the red squirrels were stationary when placed inside the ME-100 analyser, the distribution of the volume of the squirrel in the cylinder varied. EMME numbers generated on a squirrel that was lying flat and elongated in the middle of the analyzer's cylinder differed from readings made when the same squirrel was in a less relaxed posture with the abdominal region centered. Both Kenagy and Walsberg anesthetised their subjects before measuring them in the ME-100, while the squirrels in this study were simply constrained in a handling bag.

4.4.2 The ME-100 in the field

Changing fat stores as measured by TOBEC accounted for a substantial amount of the variation in spring weights of adult female red squirrels. Use of the ME-100 provided a basis for quantitative estimates of amounts of body fat stored by squirrels of different age and reproductive classes. This non-destructive method permitted monitoring of the same living individual over time, an impossibility with most other measures of body fat. As a result of this study, it was clear that weight was a good indicator of body fat so long as age and reproductive class were taken into account in experiments and in statistical analyses.

Because weight changes reflected changes in lean mass in the absence of bone growth, some squirrels may actually metabolize muscle tissue during the winter, and rebuild it in spring.

4.4.3 Annual fluctuation in weight of red squirrel females

Weights of female squirrels fluctuated with the seasons. Squirrels were heaviest in the fall during the peak of seed availability. Most females were able to sustain fall peaks in weight well into December. In January and February, females began to lose weight; lowest weights occurred in late February and March, when cached food was nearly depleted at most middens. Thus, from a bioenergetic point of view, late winter and early spring appear to be the times of greatest metabolic challenge for female squirrels. Substantial gains in weight coincided with consistently warmer temperatures in late March and April.

In late March and early April, high energy seeds are less abundant in caches, requiring squirrels to forage for cones remaining on trees (pers. obs). Despite this likely increase in the cost of foraging for seed, substantial weight gains still occurred in many non-pregnant females, probably due to lowered metabolic costs associated with the warmer ambient temperatures. Thus, spring temperatures probably play a major role in the timing and extent of weight gains of female squirrels.

4.4.4 Age differences in spring weight gain

Although they experienced similar climatic conditions, nulliparous females and juveniles were less likely to gain weight in March than multiparous females. Multiparous squirrels may maintain fat stores more efficiently due to metabolic advantages associated with their larger frame-size (Schmidt-Nielsen, 1984). Alternatively, the age related difference in weight gain may be a consequence of foraging behaviour or food supply associated with territories (young animals may be more likely to live on poor quality territories).

European red squirrels (*Sciurus vulgaris*) show annual peaks and troughs in weight similar to North American red squirrels (Wauters & Dhondt, 1989a). One significant difference between the two species is that *S. vulgaris* individuals all tend to lose weight during the spring and summer as seeds become less available. In contrast, many *T. hudsonicus* individuals gain weight in early spring before the breeding season. This difference is probably due to the social system that has evolved in each species. *S. vulgaris* rely on scramble competition and aggression over food to determine rank. Rank influences access to food, which in turn determines body condition and fertility (Wauters & Dhondt, 1989b). As food resources dwindle, all European red squirrels lose some weight because the reduction in food supply is experienced by all individuals.

Because they defend territories, *T. hudsonicus* individuals are not in continual direct intraspecific competition for food. As seed resources become depleted, the loss affects squirrels that have been unable to cache sufficient seeds in their midden more so than individuals with better caches. Body condition in *T. hudsonicus* probably depends greatly upon an individual's ability to obtain and defend a territory with sufficient food. Once a territory is secured, the ability to gain weight and maximize reproductive output probably depends upon energy efficient foraging strategies and seed production on the territory.

In conclusion, body weight is as good a predictor of body fat stores as TOBEC analysis when examining nulliparous and multiparous females. Comparisons of body fat using weight as an index should be made among squirrels of the same age, reproductive history, frame size, and during the same time of the year. Using weight as an index of body fat change is not as reliable in juvenile red squirrels as in adults because their weights are changing as a function of growth. Finally, since fertility is frequently correlated with body fat stores (Albon *et al.*, 1986), these findings strongly suggest that heavier females in each age or reproductive class should be more likely to produce a litter. Because they are able to maintain or gain high levels of body fat during the spring, multiparous females should breed more consistently than juveniles and nulliparous adults.

4.5 Literature Cited

Albon, S. D. , B. Mitchell, B. J. Huby, and D. Brown. 1986. Fertility in female Red deer (*Cervus elaphus*): the effects of body composition, age and reproductive status. *J. Zool. Lond.* (A) 209: 447-460.

Allen, W. V. 1976. Biochemical aspects of lipid storage in animals. *Am. Zool.* 16: 631-647.

Christie, W. W. 1982. *Lipid Analysis*. Pergamon Press Canada, Ltd. Toronto.

Clutton-Brock, T. H (ed.) 1988. *Reproductive Success*. University of Chicago Press. Chicago.

Eisenberg, J. F. 1981. *The Mammalian Radiations*. University of Chicago Press. Chicago.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Pond, C. M. 1984. Physiological and ecological importance of energy storage in the evolution of lactation: evidence for a common pattern of anatomical organization of adipose tissue in mammals. *Symp. Zool. Soc. Lond.* 51: 1-32.

Presta, E., J. Wang, G. G. Harrison, P. Bjorntorp, W. H. Harker and T. B. van Itallie. 1983. Measurement of total body electrical conductivity: a new method for estimation of body compostion. *American J. of Clinical Nutrition* 37: 735-739.

Price, K., K. Broughton, S. Boutin and A. R. E. Sinclair.1986. Territory size and ownership in red squirrels: response to removals. *Can. J. Zool.* 64: 1144-1147.

Schmidt-Nielsen, K. 1984. *Scaling: Why is animal size so important?* Cambridge University Press. Cambridge.

Walsberg, G. E. 1988. Evaluation of a nondestructive method for determining fat stores in small birds and mammals. *Physiol. Zool.* 61: 153-159.

Wauters L. and A. A. Dhondt. 1989a. Variation in length and body weight of the red squirrel (*Sciurus vulgaris*) in two different habitats. *J. Zool. Lond.* 217: 93-106.

Wauters L. and A. A. Dhondt. 1989b. Body weight, longevity, and reproductive success in red squirrels (*Sciurus vulgaris*). *Journal of Anim. Ecol.* 58: 637-651.

Chapter 5

The Timing of Estrus in Red Squirrels: a Search for Proximate Mechanisms

5.1 Introduction

The timing of reproduction can influence the survival of both parents and offspring and is thus critical in an evolutionary sense (Baker, 1938). Few field studies have attempted to define the factors responsible for individual variation in timing of reproduction, other than in terms of presumed ultimate evolutionary advantages (Ims, 1990, McShea, 1989). Bronson (1989) notes that except for a handful of laboratory and domestic species such as hamsters, sheep, cats, and rats, the proximate mechanisms regulating onset of breeding are poorly understood in mammals.

To understand the degree of synchrony in reproductive events, it is important to determine the ways in which proximate environmental conditions influence temporal breeding in many species (Ims, 1990; Bronson, 1989; Negus & Berger, 1987). Asynchrony in reproduction may result from variation in the proximate stimuli available to individuals, from variable responses by individuals to proximate stimuli, or from a combination of the two. The aim of this study was to determine some of the intrinsic and extrinsic factors responsible for temporal variation in reproduction in red squirrels.

Female red squirrels exhibit relatively great variation in the timing of estrus both within a given year and between years (Rusch and Reeder, 1978). Abundant cone production in the fall has been associated with relatively early breeding in the following spring (Smith, 1968; Millar 1970; Rusch and Reeder, 1978). These authors speculate that year to year variation in the onset of red squirrel breeding seasons depends upon energy available throughout the winter as a consequence of the fall cone crop. During a single breeding season, the time elapsing between estrus dates of different individuals may be as great as two months in some boreal *Tamiasciurus* populations (Millar, 1970; Rusch and Reeder, 1978). Ground squirrels and other tree squirrels that produce one litter per season tend to have a smaller range in estrus dates: 14 -29 days (Heaney, 1981).

The proximate mechanism controlling the onset of estrus in red squirrels is unknown (Lair, 1985), and no specific hypotheses appear in the literature addressing the cause of the relatively large spread in estrus dates found in this species within a given year. By manipulating the amount of food available to individuals and making predictions about the consequences, I have tried to discern the ways in which food and costs of foraging affect the timing of estrus in female red squirrels, a territorial, food-hoarding mammal. This paper focuses on three main questions about the timing of reproduction in red squirrels:

1. What factors determine the average timing of estrus in the population from year to year?
2. What factors affect the "spread" of estrus events around the average?
3. What factors determine the timing of estrus on an individual basis?

5.1.1 Hypotheses and predictions about average timing of estrus from year to year

There are at least two broad, alternative hypotheses regarding extrinsic environmental control over timing of reproduction: (1) variation in timing depends on a physical cue (dietary non-energy plant substance or ambient condition) or (2) variation in timing depends on net energy gain (the caloric balance between net food energy obtained and the costs associated with survivial). The first hypothesis, cue variation, suggests that timing of reproduction depends upon a proximate stimulus that varies in time. For example, Rusch and Reeder (1978) suggested that patches devoid of snow are required for squirrel courtship. Dietary cues other than caloric balances were proposed by Lair (1985) and by

Kemp and Keith (1970). Specifically, they suggest that non-energy nutrients in the buds of conifers may trigger estrus in squirrels.

The "net energy gain" hypothesis is derived from a combination of Bronson's (1985) suggestion that "annual patterns of breeding reflect the caloric costs of the female's reproductive effort as they relate to the costs and gains associated with her foraging effort", and the "over winter energy" hypothesis, that abundant cone production in the fall causes advanced breeding in the following spring (Smith, 1968; Millar 1970; Rusch and Reeder, 1978). This hypothesis states that average timing of estrus is determined by the net energy gain to individual squirrels during the breeding season.

In contrast to these two hypotheses for extrinsic environmental control, it is equally possible that individual variation in endogenously "programmed" timing is great enough to account for the seasonal patterns previously described.

These three main hypotheses result in mutually exclusive predictions. Two predictions follow from the net energy gain hypothesis. First, timing of estrus should be earliest in years having low metabolic costs and/or high gains, while late dates of estrus should occur under the opposite environmental conditions. Specifically, if winter and spring temperatures are mild and follow a peak cone crop, estrus dates should be relatively early. Low food abundance combined with low temperatures in spring should cause squirrels to breed later in the year. Secondly, additional food should advance the average dates of estrus in red squirrels.

A mechanistic hypothesis related to the above idea is that an energy threshold may regulate the timing of reproduction (Bronson & Manning, 1991). Food addition may allow individual squirrels to surpass critical energetic thresholds earlier in the season, thus advancing ovulation and conception. If this is the case, early weight gain in spring (positive energy balance) should correlate with early estrus and births (Section 4.4.4).

If food addition fails to shift the average dates of estrus, then squirrels are probably reliant on other environmental cues (i.e. nutrients in buds) or on intrinsic (Kenagy 1980, 1981) endogenous circannual rhythms (section 3.1)

5.1.2 Hypotheses about asynchrony of estrus

Three potential hypotheses for the asynchrony or "spread" in estrus dates exhibited by squirrels can be tested. The first hypothesis is derived from the net energy gain argument, while the other two are alternatives, but are not necessarily mutually exclusive.

1. Timing of estrus varies because females must expend different amounts of energy to obtain food.

    Prediction: If variation in net energy gain causes asynchrony, then food addition should synchronize estrus, by reducing handling and foraging time differences that exist between individuals.

2. Individual traits of female squirrels (possibly genetic) causes asynchrony.

    Prediction: If individual variation is a strong factor, food addition will not synchronize estrus.

3. Asynchrony in estrus results from the age structure of females in estrus.

Younger, first time breeders increase variance in estrus dates.

> Prediction: The more primiparous females (first time breeders) in the population, the greater the asynchrony will be during the breeding season.

5.2 Methods

The breeding season was defined by dates of estrus in female squirrels. Because most squirrels were monoestrous and conceived within a day or two of estrus, date of conception was considered the same as date of estrus. Yearly differences in the breeding seasons were defined by differences in the means and ranges of single estrus (conception) dates. Changes in means, standard deviations and ranges of Julian dates of estrus were used to measure the effects of additional food on the timing of estrus.

5.2.1 Study Site

Research was conducted over a three year period (1988-90) in the Athabasca River Sandhills southwest of the village of Fort Assiniboine in central Alberta (54°17' N, 114°50' W). The study site consists of fragmented islands and peninsulas of pine stands on top of ancient sand dune formations (David, 1977). These pine habitats are surrounded by aspen parkland in the uplands, and boreal bogs in depressions. Jack pine (*Pinus banksiana*) is the dominant conifer on most of the squirrel territories. Black spruce (*Picea mariana*), white spruce (*Picea glauca*) and larches (*Larix laricina*) are present in small patches on lowland territories.

5.2.2 Climatic data and estimates of annual cone crops

Daily minimum and maximum temperatures for the site, provided by the Alberta Forest Service in Fort Assiniboine, were used to calculate mean daily and monthly temperatures.

Annual cone productivity in jack pine was estimated in two ways. First, cones produced in a specific year and harvested by squirrels were examined for bunches containing 2, 3, 4, 5, and 6 attached cones. Squirrels seem to prefer the mature, green jack pine cones produced each fall. It was assumed that bunch size increased during years of high cone production and that the average bunch size collected by squirrels could be used as a relative index of cone production. Green cone bunches were tallied at 15 middens in

September of each year. The sampling technique consisted of filling a five gallon bucket with cones (about 1000 individual cones) and determining the average bunch size.

The second method was to count green cones produced on a sample of trees each year. In August of 1988, 89 and 90, branches (N=40) from a selection of jack pines were examined, and the number of green cones on each branch was tallied as an index of cone production (Larsen, pers. comm.).

Cones remaining on top of squirrel middens in mid-March were assumed to reflect a combination of availability in the fall and use during the winter. Total counts of cones cached above ground at the midden were used as a relative index of cached seeds available to squirrels in spring. A qualitative evaluation of cones remaining on trees was made by looking at the upper third of several trees and recording "none" when no cones were seen, "very few" when less than about 50 cones were seen, "few" for 50 to 100 cones, "moderate" for 100 to 200 cones, and abundant for more than 200 cones. Counts were made in mid-March in 1988 and in late May of 1989. No qualitative assessment of cone availability on jack pine trees was made in 1990.

5.2.3 Addition of food to individual squirrels

During the breeding season 1988 and throughout the winter and breeding season of 1988-89, weight changes, timing of proestrus, and timing of estrus and birth were recorded for 65 female red squirrels. Thirty females received a renewable cache of sunflower seeds as described below. In the winter and breeding season of 1989-90, the caches of 12 juvenile females were supplemented during winter and spring.

Sunflower seeds were provided *ad lib* in a large bucket at each squirrel's midden. The buckets were sometimes buried into the midden to prevent chickadees, gray jays and other mammals from easy access to the seeds. Thirteen squirrels received seed additions throughout the winter and spring of 1989, while 17 females (9 in the spring of 1988 and 8 in the spring of 1989) received seed additions just before the breeding season. Winter seed additions were continuous on each treatment territory from October 31 to parturition of each female. Spring seed-additions were made on February 14 in both years and were maintained until parturition or June 15, whichever came first. In 1989, sunflower seed consumption (grams per day) was measured by monitoring changes in the volume of seeds in the buckets.

5.2.4 Trapping, handling, weighing and timing reproductive events

During the winter of 1988 (December-February) and fall and winter 1989 (October-February) squirrels were trapped every other week. Once proestrous vulva-swelling was noted (February-March), females were caught and measured at 3-4 day intervals, until date of estrus was determined. Measurements were made less often during the third field season (October 1989 - July 1990), but the sample size of females studied was increased from 20 (1987-88) and 45 (1988-89) to 108.

To catch the squirrels, Tomahawk live-traps baited with peanut butter were set at the middens used female squirrels. From the trap, a squirrel was transferred to a cone-shaped cloth bag, in which weight and reproductive status were determined. The zygomatic width (mm) of a squirrel was measured during each trapping session to monitor growth, and to provide an assessment of body size independent of weight.

Vaginal smears, vulvae swelling and mating chases were used as indicators of dates of proestrus and estrus (Millar, 1970; Modafferi, 1972; Smith, 1968). When an estrus date had been determined, each female was trapped infrequently until near the expected date of parturition, at which point trapping was resumed. Sudden loss of weight (15-20% of total body weight) and lactation signaled that birth had taken place.

If the location of the squirrel's nest was not obvious, the lactating female was radio-collared and tracked to the nest. The offspring were weighed, and these data were used to backdate to birth and estrus. The dates of estrus calculated by backdating from known dates of parturition were standardized using an average gestation length of 37 days. This mean was calculated from 14 cases of known gestation length: sperm were present in an estrus smear and mating chases had been seen on that day, and the female and her litter were captured on the day that she bore her young.

For backdating, squirrel growth rates determined from 4 litters raised in a captive setting were used to calibrate the relationship between a squirrels age and its weight. The relationship between days elapsed since birth (y) and squirrel weight (x) for 11 squirrels was strongly linear (r^2=0.96, p= 0.0001). The equation for the linear fit was $y = 0.413 x - 0.83$.

Three age or reproductive classes of female squirrels were recognizable in the fall. Adult females that had nursed at least one litter in a previous season (multiparous) and those who had not (nulliparous), were determined by nipple condition (Layne,1954). Juveniles (females less than a year old) were recognized by extremely low fall weights, lack of fully-furred tails, and small zygomatic widths. In the spring, females giving birth to a litter for the first time were referred to as primiparous females.

5.3 Results

5.3.1 Timing of Breeding: general patterns in female squirrels on a normal pine seed diet

Variation in average timing and degree of asynchrony in conception dates during the three breeding seasons was determined for female squirrels that were not given any additional food. The mean date of conception in 1988 was statistically earlier than dates in 1989 and 1990, by 8 to 11 days (ANOVA, $F_{2,98}$=5.1, P= 0.0079). In 1988, the breeding season began the first week of March, while in 1989 the first matings occurred during the last week in March. In 1990, the earliest successful mating occurred during the second week of March. The mean date of estrus in 1988 was April 1 (Julian date= 91± 14), while the means in 1989 and 90 were April 9 (Julian date= 99 ± 12) and 11 (Julian date= 100 ± 11) (Figure V-1).

During the two seasons with a similar mean estrus date (1989 and 90), the spread in estrus dates increased with the proportion of first time breeders. The proportion of litters from primiparous females in 1990, a year in which estrus dates occurred over a 9 week season, was 42% (N=43). In contrast, litters produced by first time breeders in 1989 made up only 15% of all litters, and this season lasted only four weeks (Figure V-1). In 1988 no more than 22% of the females were primiparous, but the season was still 9 weeks long.

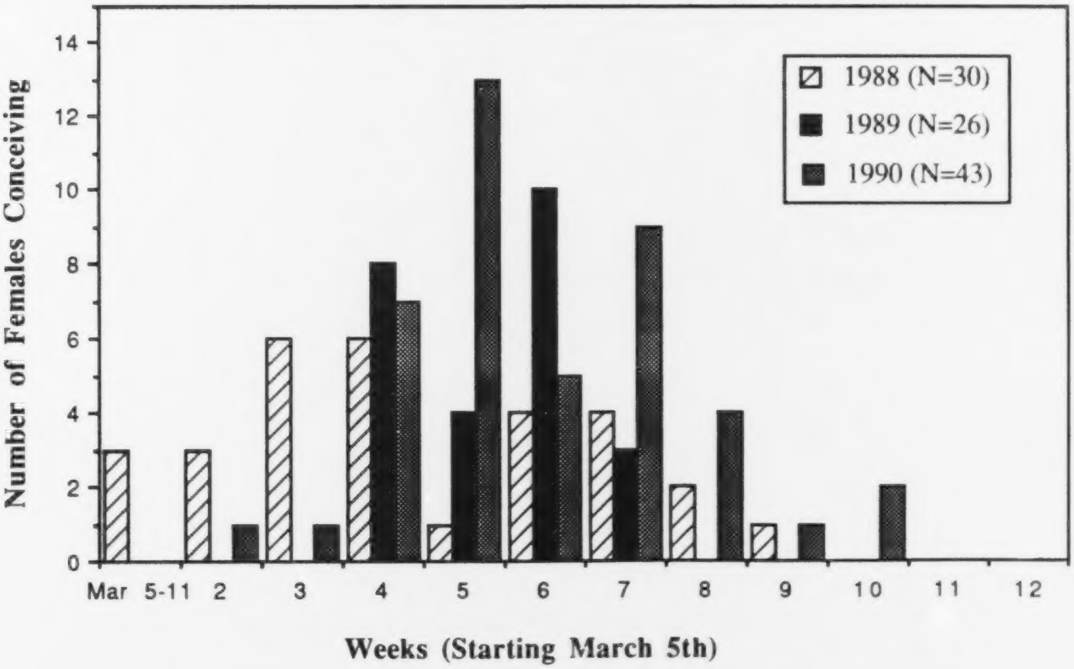

Figure V-1: Variation in the timing of estrus in an Albertan red squirrel population (1988, 1989 and 1990).

In 1990, the mean date of estrus was later in primiparous females than in multiparous females (t=2.3, d.f.=41, p= 0.012). No significant difference in timing was noted between these two reproductive classes in the breeding seasons of 1988 and 1989 (Table V-1). The standard deviations around the mean estrus dates were largest in 1988.

Table V-1: Mean date of estrus (Julian days) by year and reproductive status for squirrels that produced litters.

Year	N (Primiparous)	Mean ± s.d.	N (Multiparous)	Mean ± s.d.
1988	6	90 ± 20	23	91 ± 16
1989	4	95 ± 6	22	99 ± 13
1990	18	106 ± 14	25	98 ± 11*

* t = 2.3; d.f.=40, p= 0.012

5.3.2 Climatic patterns and cone production in jack pines

The winter and early spring months (January-March) of 1989 were colder on average than the same months in 1988 and 1990 (ANOVA, $F_{2,265} = 4.84$, $p < 0.008$). When compared to 10 year averages, the early spring of 1988 (February & March) was warmer than average. Mean temperatures in 1990 were colder than the ten year mean in February, but warmer than average in March (Figure V-2). In summary, 1988 had the mildest winter and spring, while 1989 was coldest and 1990 was intermediate.

Fall estimates of jack pine cone production on 40 branches suggest that the cone crop of 1989 was about 15% larger than the crops in 1988 and 1990 (Table V-2). Analysis of bunches of cones harvested in the fall suggested that the 1987 cone crop was smaller than the crops of 1988 and 1989. The mean bunch size in 1987 was half that of 1988 and 1989 (Table V-2).

Mean cone abundance on middens around the time of the spring equinox was highest in 1990 and lowest in 1989. Values in spring of 1988 were closer to those of 1989, despite the mild winter conditions of 1987-88 (Table V-2).

More trees were rated as having abundant or moderate numbers of cones in 1989 (15 of 25 locations) than in the spring of 1988 (5 of 25 locations). In 1988, nine locations were rated as having "very few" cones, whereas in 1989 only one of the same 25 locations received that rating ($\chi^2 = 18.7$, d.f.=6, $p = 0.005$). The combination of all the cone crop information suggests that fall cone production was lowest in 1987, intermediate in 1988, slightly higher in 1989 (Table V-2).

Table V-2: Cone crops and winter climate patterns 1987-90 (Athabasca Sandhills, AB)

A. Fall Cone Crop Assessments

Year	Branch count	Cones/bunch (mean)	Qualitative Production
1987	unknown	2.3 ± 1.2 (N=212)	Light
1988	118	4.2 ± 2.2 (N=245)	Moderate
1989	137	3.8 ± 1.8 (N=300)	Moderate to heavy

B. Spring Cone Assessments

Year	Mean no. of cones/midden ± 1 S. E.	Winter & Spring Climate
1988	98 ± 29 (N=19)	Very mild
1989	84 ± 16 (N=60)	Very cold
1990	140 ± 23 (N=98)	Cold

Table V-3 summarizes relationships between aspects of the three breeding seasons, bioenergetic conditions in spring and the numbers of females breeding for the first time in that season. In the breeding seasons of 1988 and 1989, relatively poor bioenergetic conditions in spring were associated with a reduction in the number of primiparous females. In 1990, bioenergetic conditions favored high net energy gain in the spring and a relatively large number of females were primiparous. That year, moderate spring temperatures followed a fairly heavy cone crop, and as a result, large numbers of cones were still available at squirrel caches in March. A bimodal, reproductive class-dependent distribution in estrus dates was noted in 1990.

When the breeding population was dominated by multiparous females in 1988 and 1989, the relative abundance of cones remaining on trees was associated with the degree of synchrony in estrus dates. In 1989 estrus dates were more synchronous than in 1988, and there were more cones available on trees. In spring of 1988, temperatures were extremely mild, but because of the light cone crop, food was less available. Many more middens appeared to be totally depleted of cones in 1988 than in the following two years. Although annecdotal, squirrels seemed to feed on buds and cambium of jack pines more so in the spring of 1988 than in the other springs. In 1988 there were five middens that were totally depleted of cones, their below-ground caches excavated and emptied by the resident squirrel. At the same time in the following year, surface cones at the same five middens numbered over 100 and below ground caches had not been excavated.

Table V-3. Factors associated with the breeding seasons of red squirrels. Coefficients of variation are given as a percentages (CV %).

Breeding Season Traits				Factors associated with breeding season		
Year	Length	Onset	CV %	Spring	Cone crop*	% primiparity
1988	9 wks	earliest	16.1	mild	light	22%-low
1989	4 wks	latest	12.6	very cold	medium	15%-low
1990	9 wks	ave.	11.0	moderate	med. heavy	42%-high

* cone crop in previous fall

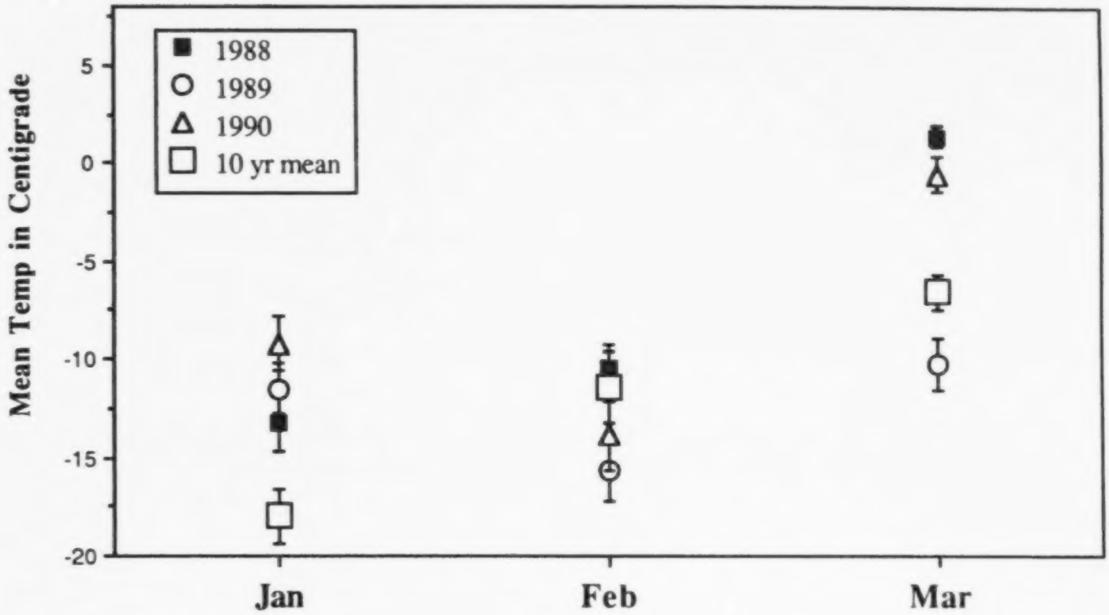

Figure V-2: Mean temperature °C ± 1 S.E. in January, February and March (1988, 1989 & 1990 and 10 year average) at Fort Assiniboine Forest Station, Alberta, Canada.

5.3.3 Effects of seed addition on timing of estrus in experimental groups

The estrus dates of squirrels given sunflower seeds during the spring of 1988 were not significantly earlier than among the unmanipulated squirrels. The ranges of estrus dates (asynchrony) were smaller in the treatment groups than in the control groups of female red squirrels (Table V-4).

All the sunflower seed additions given to affect the breeding season of 1989 caused advancement of mean estrus dates (Figure V-3). Unmanipulated squirrels did not come into estrus until the end of March, while females given seed-additions cycled in early to mid-March of 1989. Seed additions present throughout the winter and spring advanced the mean dates of estrus in multiparous and nulliparous females (two-factor ANOVA, $F_{1,27}$

=64.9,p= 0.0001). As shown in Table V-4, females given sunflower seeds during winter and spring had a mean estrus date three to four weeks earlier than unmanipulated squirrels (ANOVA, $F_{1,29}$ =73.6, p= 0.0001). Females that were given sunflower seeds only in spring had estrus dates averaging 10 days earlier than squirrels feeding on a pine seed diet . The earliest estrus date of the year, February 23, was from a multiparous female given sunflower seeds throughout winter and spring.

Table V-4: Effect of sunflower seed-additions on dates of estrus in red squirrels.

1988 Spring seed-addition

Group (N)	Range of Julian Estrus dates	Mean Estrus date ± s.d.	Effect
Control (8)	89 to 133	110 ± 17	not significant
Seed adds (8)	86 to 119	101 ± 12	

1988-89 Winter and spring seed-addition

Group (N)	Range of Julian Estrus dates	Mean Estrus date ± s.d.	Effect
Control (18)	86 to 145	103 ± 8	p= 0.0001
Seed adds (13)	57 to 87	77 ± 9	

1989 Spring seed-addition

Group (N)	Range of Julian Estrus dates	Mean Estrus date ± s.d.	Effect
Control (18)	86 to 145	103 ± 8	p= 0.0001
Seed adds (6)	83 to 101	91 ± 9	

Seed addition synchronized estrus dates in adult multiparous females. Females (mainly multiparous) on a normal diet of conifer seeds ("controls") had a nine week range in estrus dates (Figure V-3). In contrast, estrus dates in multiparous females feeding on sunflower seeds ranged within a five week period. When all the animals given sunflower seeds are combined, their breeding season covered an eight week period. By artificially increasing the number of first time breeders, additional food made the total range in estrus dates as large as the control group in the 1990 season.

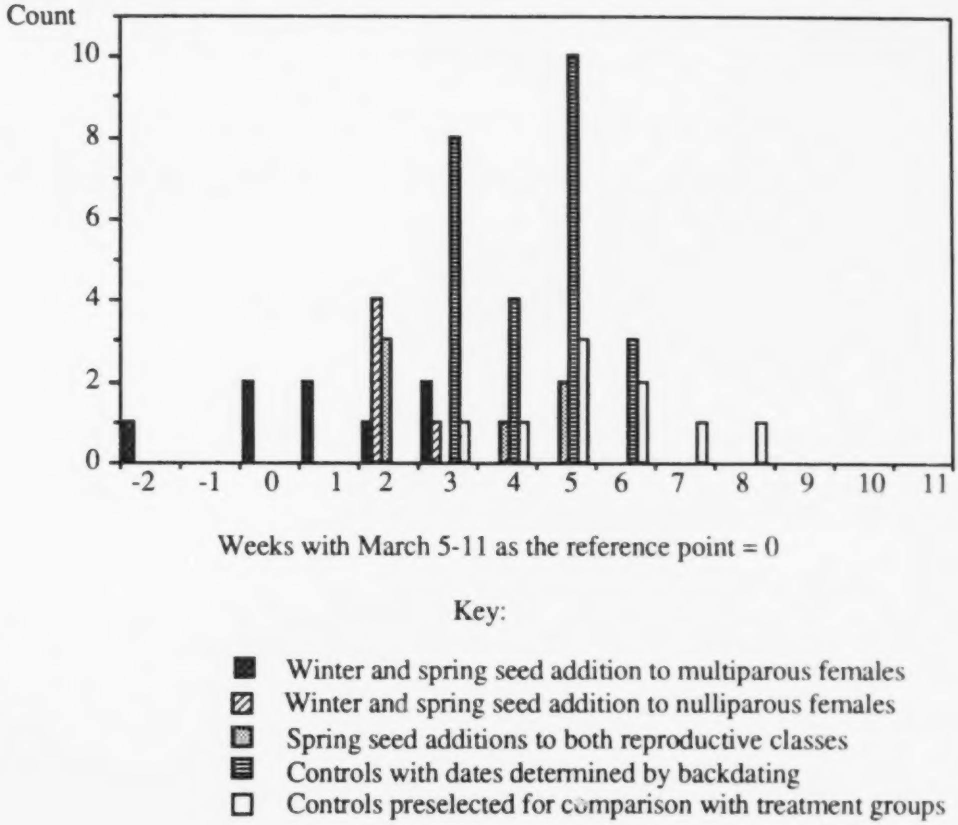

Figure V-3. Timing of estrus and conception in groups of female red squirrels in response to food addition and as a function of reproductive status during the breeding season of 1989.

Sunflower seed caches provided throughout the winter and spring caused greater advancement in estrus dates in multiparous females than in nulliparous females (Figure V-3). When compared with controls in the same reproductive class, the mean estrus date was advanced by 27 days for multiparous females, but only by 17 days in potential first time breeders.

In 1990, juveniles provided with sunflower seeds had estrus dates that were 14 days earlier, on average, than those of primiparous adults on a natural diet. (In the control group, only one of 35 juveniles cycled and conceived, so timing comparisons were made with primiparous adults). Mean Julian date of estrus for the juveniles given sunflower seeds was 99 ± 11 (N=11) and in unmanipulated primiparous squirrels the mean Julian date of estrus was 113 ± 15 (N=18) (t=2.28, df=27, p= 0.03). Seed addition also synchronized the estrus dates. The range of dates of the control group spanned a two month period (59 days), while the range in the sunflower seed-addition group spanned 32 days.

5.3.4 Timing of estrus and consumption of seeds

Estrus dates for females that fed on sunflower seeds throughout the winter of 1988-89 were negatively correlated with consumption rates: greater consumption was linked with advanced estrus (Figure V-4).

5.3.5 Effect of winter seed-addition on weights and timing of reproductive investment

Analysis of Covariance (ANCOVA) with repeated measures was used to compare the effects of sunflower seed additions on mean weights obtained during winter and spring before breeding. Significant differences existed between mean "start" weights of nulliparous versus multiparous squirrels. However, there was no significant reproductive class effect on weight gain or loss in the repeated measure part of the model. The effect of seed addition on timing and variation in weight gain was significant in both reproductive classes (ANCOVA with repeated measures, $p = 0.009$). Both nulliparous and multiparous red squirrels that fed on sunflower seeds maintained higher winter weights than control squirrels. In addition, the squirrels given sunflower seeds increased in mean weight earlier in the year than the control squirrels. The latter lost weight during the early spring of 1989 and showed an increase in mean body weight later in the same spring (Figure V-5).

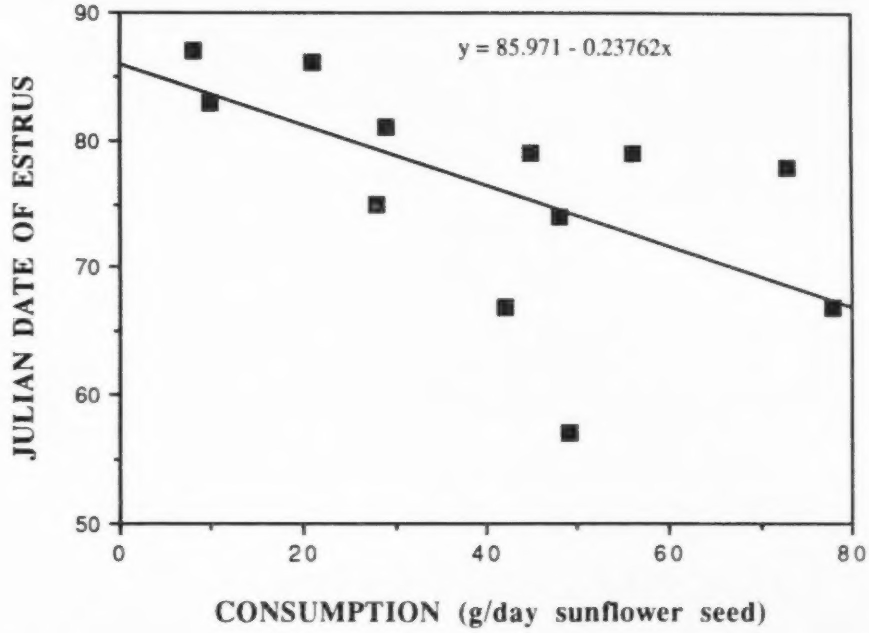

Figure V-4. Linear relationship between daily consumption of sunflower seeds and timing of estrus. Higher rates of seed consumption by female squirrels were correlated with earlier dates of estrus ($r^2 = 0.38$, $P < 0.05$).

Because the ANCOVA samples were relatively small, due to missing animals in a given trapping session, the time of weight gain in a larger subset of squirrels was analysed for variation explainable by reproductive experience and treatment. The ANOVA results were similar to the ANCOVA results. Seed addition treatments (two-factor ANOVA, $F_{1,35}=5$, p

= 0.03) and reproductive experience (two-factor ANOVA, $F_{1,35} = 5.82$, $p = 0.02$) influenced the timing of weight gain in female squirrels. Table V-5 shows the mean Julian dates of gain in weight. The date recorded is the first in which the record that follows shows at least a 10 gram increase above the lowest weight measured.

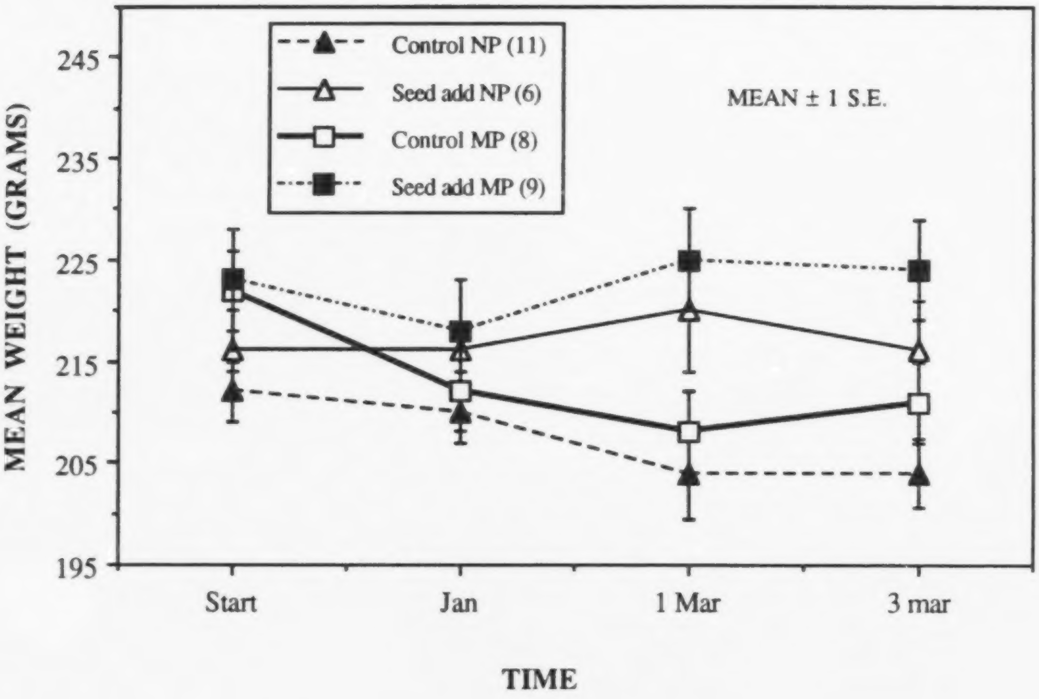

Figure V-5. Plots of mean body weight ± 1 S.E. of female squirrels as a function of treatment, reproductive status and time. Repeated measures ANCOVA using November weight as a "start" value (the covariate) showed that seed addition reduced winter weight loss and resulted in an earlier weight gain in adult female squirrels. The pattern of weight change was similar in nulliparous (NP) and multiparous (MP) red squirrels. (1 Mar refers to measurements made during the first week of March, while 3 Mar refers to the third week in March).

Table V-5. Timing of spring weight gain in female squirrels (1989)

Reproductive Status	N	Treatment	Mean Julian date of gain ± 1 s.d.
Multiparous	8	Seed Add	60 ± 17
Multiparous	10	Control	76 ± 22
Nulliparous	9	Seed Add	77 ± 10.5
Nulliparous	12	Control	84 ± 11

The mean timing of spring weight gain was weakly correlated with the mean timing of estrus in all of the female squirrels in 1989 ($r^2 = 0.26$, d.f.= 33, $p = 0.002$). Multiparous females with seed additions were first to gain weight and first to ovulate. Multiparous female controls showed seasonal gains at about the same time as the nulliparous females given seed additions, but they did not ovulate as early (Table V-6). Nulliparous control females were last to show weight gains, but the distribution of their estrus cycles was similar to the multiparous controls.

Table V-6. Order of weight gain and Julian dates of estrus in the 1989 breeding season

Order of spring gain	Status	Treatment	JD estrus ± 1s.e.	N
First	multiparous	seed add	77 ± 4	10
Second (tie)	multiparous	control	103 ± 2.4	11
	nulliparous	seed add	86 ± 3	9
Fourth	nulliparous	control	102 ± 3	7

5.3.6 Do spring weight and weight gains influence timing of conception in unmanipulated squirrels?

Early weight gains and high average spring weights seem to correspond with early estrus in the seed addition groups. If body condition alone acts as a threshold for timing ovulation, the same pattern should exist in unmanipulated squirrels. This did not appear to be the case. In the 1988-89 breeding season, the Julian date of first gain showed no predictive relationship with estrus date (r^2=0.111, d.f.=16, F=1.8, p=0.191). Average spring weight also had no significant relationship with timing of estrus (r^2=0.131, d.f.=17, F=2.42, p=0.14). Again in 1990, spring weight had no statistical relationship with date of estrus in the control group (r^2= 0.06, d.f. =28, F=1.8, p= 0.19).

The weight gain sub-hypothesis predicts that early estrus may be caused by attainment of high weight (body fat levels) early in the breeding season. Because weight is a good predictor of body fat (Section 4.3.1) and zygomatic breadth is a good measure of body frame size, a regression of these two factors provides a relationship with which to estimate body fat.

The linear relationship between March weight in grams (y) and zygomatic width in mm (x) for 82 female squirrels (the 1990 population) was $y = 15.53x - 235$ (r^2 = 0.492, F=79.3, p= 0.0001). Fat levels were estimated by subtracting expected weights (values of March weight generated by substituting real values of zygomatic widths into the equation) from observed weights (weight value measured in the field). When fat estimates (grams of fat) were regressed on 1990 estrus dates the linear relationship (Julian estrus date = 0.3 fat (g)+ 102) was almost significant (r^2=0.18, d.f.=17, F=3.56, p= 0.0773). However, the slope was in the opposite direction from that predicted by the hypothesis. The trend suggested that fatter squirrels were breeding later in the season than thin, light ones.

5.3.7 Are individuals consistent in timing of estrus?

Seven multiparous females that were given seeds in 1989 were monitored as controls in 1990. Because there were no differences in mean estrus dates of the control population in the two breeding seasons, the effect of seed addition on a given individual's estrus date could be compared. The comparison also provided a test of individual consistency in timing from year to year.

In all cases, estrus dates were earlier when the female received sunflower seeds than when she was in a control group. On average, an individual's estrus occurred three weeks earlier than normal in response to sunflower seed addition (paired $t=-8.2$, $df=6$, $p < 0.045$). Standard deviations were similar: 10.3 days with the sunflower seed additions and 9.8 days without. Regression of the 1989 estrus dates against those of 1990, indicated that individual variation in timing was fairly consistent from year to year. Individual consistency in timing explained 63% of the variation in this sample of adult female squirrels ($r^2=0.63$, $df=6$, $F=8.57$, $p< 0.033$).

5.4 Discussion

5.4.1 Average timing of estrus

Addition of food at the middens of female squirrels caused advancement of the average date of estrus, conception and consequent births. Both the advancement in the mean estrus date of a treatment group in a single season and individual advancement of estrus in response to seed additions in different years show that reductions in the cost of obtaining food energy plays an important role in timing of estrus.

Despite following a fall of relatively light cone production, the 1988 breeding season was initiated earlier than the next two seasons that followed relatively heavy cone crops. Reduced metabolic costs caused by warm spring temperatures seem to be more important in advancing the squirrel breeding season than the size of the cone crop. This finding refutes the premise in the "over-winter energy hypothesis", that cone crops function as the major factor controlling the timing of squirrel breeding seasons (Smith, 1968; Millar 1970; Zirul,1970; Rusch and Reeder, 1978). Still, annual fluctuation in the average timing of estrus dates was influenced directly by a combination of food availability and spring temperatures. These results support the net energy gain hypothesis and refute the idea that average timing of estrus is due to intrinsic random variation.

Rusch and Reeder (1978) suggested that open patches of ground were required for courtship and mating in red squirrels, because they found that the time elapsed from January 1 to dates of major snowmelt correlated well with the onset of breeding. Although Lair (1985) refuted this with observations of mating chases when snow still fully covered the ground (as were seen in this study as well), the obvious relationship between temperature and snowmelt corroborate bioenergetic explanations for timing of breeding. When snowmelt is early, metabolic costs of foraging are probably reduced earlier in the year. When benefits of foraging consistently outweigh the costs, fat deposition is possible. A consistent positive caloric balance may cue the females' reproductive endocrinology towards ovulation once reproductive readiness has been achieved (Section 3.3).

The results of the seed addition experiments refute the hypothesis that external cues like nutrients or phytoestrogens in buds play a role in timing estrus other than via a caloric pathway. If squirrels depended on these other cues, the seed additions should have shown no effect at all. Instead, the seed additions advanced the average timing of estrus.

Estrus did not occur at the time of the seed-additions, so reproductive readiness was not triggered by caloric or nutrient intake *per se*. The absence of an external or internal primer in conjunction with appropriate bioenergetic conditions seems to prevent ovulation from occurring any earlier than mid-February. This primer is likely to be an endogenous rhythm of reproductive readiness that is entrained by day length (see Chapter 3). Nevertheless, the strong results of the food addition experiments and the interrelationship between cone crop size, mild winters and early breeding suggest that the mechanism for timing estrus (once a squirrel is reproductively ready) depends on the individual's sensitivity to net energy gain.

5.4.2 Asynchrony

In both the control and experimental groups, the degree of asynchrony or range in estrus dates were directly influenced by energy availability and ambient temperatures in spring. Secondarily, the length of the season was influenced by the proportion of first time breeders in the population. The breeding season was most synchronous when the percentage of primiparous females contributing litters was low and food availability was relatively good. This occurred when the cone crop prior to breeding was moderate in size and spring temperatures were colder than average (1989). The range in estrus dates was greatest either when the proportion of first-time breeders was high (1990) or when multiparous females were affected by a cone shortage (1988). These patterns suggest that extremes in net energy gain in the spring increase the range of estrus dates. When bioenergetic conditions and consequent net energy gains are "very poor" or "very good" asynchrony is large, but when bioenergetic conditions are "intermediate" (adequate food, but cold) asynchrony is low. This pattern can be described by plus and minus ratings for cone crops and spring temperatures in terms of net energy gain (Table V-7).

Table V-7. Summary of variables affecting net energy gain in a positive (+) or negative (-) direction, and their combined outcomes in terms of timing and asynchrony of estrus dates in red squirrel breeding seasons (the -,- relationship is speculation).

Cone Crop	Spring Temperature	Timing of the season	Asynchrony in the season
+	+	very early	very asynchronous
+	-	late	most synchronous
-	+	early	asynchronous
-	-	very late	very asynchronous

5.4.3 Age effects

No matter how favorable bioenergetic conditions were, age still caused asynchrony in estrus and births in control and treatment groups. When the proportion of primiparous females was large in control groups in 1990, the first time breeders had average estrus dates that were later than those of multiparous females. The same result was created when previously nulliparous squirrels were given *ad lib* sunflower seeds in 1989. These results suggest that juveniles, and primiparous adult (relatively young) females are bioenergetically disadvantaged or constrained in comparison to multiparous (relatively old) females. Perhaps with age and experience, a female either becomes more efficient in obtaining energy on her territory, or simply obtains a better territory. Removal experiments done by Larsen *et al.* (pers. comm.) at the same study site found that females rarely abandon their territories for vacant ones. Thus improvement in foraging efficiency is fairly likely. In addition, other metabolic costs associated with youth (such as growth) may compete for energy and prevent early reproduction in first time breeders, particularly in juveniles.

Seed additions sychronized timing of estrus, but not to the extent observed in animals with endogenous rhythms or obligate responses to photoperiod. For example, one would expect to reduce the spread of estrus dates to about 10 days, the amount of variation in timing in ground squirrels and hibernators with endogenous reproductive cycles (Murie, pers. comm.) Even with an attempt to standardize energy and nutrient availability, individual dates of estrus spanned a one month period. Variation in timing of estrus was quite consistent in the comparison of the same individuals in two different years, suggesting that individual sensitivity to net energy gain is an important factor affecting the timing of estrus in red squirrels. In the control groups, fatter squirrels tended to breed later in the season, suggesting that variation in body condition thresholds might cause asynchrony. In as much as high seed consumption rates were correlated with earlier estrus dates, individual feeding behavior could also influence timing of weight gains.

5.4.4 Potential evolutionary significance of timing patterns

The persistence of asynchrony and individualized timing of estrus in red squirrels is of theoretical interest because it supports McShea's (1989) hypothesis that ultimate benefits may select for asynchrony. McShea suspects that the asynchrony in territorial microtine rodents is favored by natural selection, since staggering births allows space partitioning that is more temporally efficient among females. The same argument could apply to temporal acquisition of territories by juvenile squirrels. If females produce their young at the same time, the young may experience greater intraspecific competition when they are weaned and are vying for space during dispersal. Nevertheless, it is equally possible that the pattern is based on random genetic variation that has not been fine-tuned by any ultimate costs or benefits (eg. predator swamping).

The ultimate advantages associated with advancing the average timing of estrus are more obvious and less debatable than those suggested for the evolution of asynchrony. Within a given year, early breeding should be favored in red squirrels because territories are limited, and obtaining one greatly increases an individual's probability of surviving (Boutin, pers. comm.). Thus, juvenile squirrels that are conceived and weaned first should face the least amount of competition from conspecifics for vacant territories. Field studies in Yukon spruce habitat support the prediction that squirrels born early are more likely to obtain territories (away from their natal one) than squirrels born late in the summer (Price, 1989). Some females that breed late in the season "bequeath" their territories to their offspring, presumably to buffer them against the poor odds of securing a territory late in the season (Price, 1989).

In addition to enhancing juvenile survival, early estrus should be favored in red squirrels because they are physiologically capable of producing a second litter. Early breeding may give the female squirrel the time needed to produce two litters when conditions permit. A second litter was produced by three different females during the study. Two of these females had surplus food, while the third case was for a control female that bred early in the season. Early breeding is therefore both theoretically and empirically well-coupled with high natality in red squirrels.

5.4.5 Handling time, net energy gain and seasonal breeding

Variation in average timing of estrus (conception) among female red squirrels was caused by differences in net energy gain after they were reproductively ready (had shown proestrus vulva swelling). For each female given the sunflower seeds, energy intake apparently exceeded some critical value of metabolic cost earlier in the season. Exactly how increases in net energy gain and consequent increases in body fat translate into cueing estrus is a physiological problem beyond the scope of a field study, but this seems to be the underlying mechanism for timing ovulation.

Reductions in handling and foraging time are the obvious physical reasons for enhanced body condition of squirrels given the seed additions. Red squirrels in jackpine forests are relatively constrained by their food resources. Each adult red squirrel defends an exclusive territory, averaging about 0.4 hectares, from which it obtains food (Gurnell, 1984; Larsen, pers. comm.). Squirrels save substantial amounts of energy by harvesting cones in the fall when foraging costs are low, and avoiding foraging costs during winter by using the cache. Cached jack pine cones, the main winter and spring food source of the squirrels, are still relatively costly because of handling time. On average, an adult squirrel takes four minutes to extract and eat the seeds from one jack pine cone. This provides the squirrel with about 2 kJ of energy (Smith, 1968), or 0.4% of its daily energy requirement (assumes a 220 gram non-lactating, non-pregnant squirrel). By eating two sunflower seeds, a squirrel can obtain the same amount of energy in less than 20 seconds, a twelve-fold decrease in handling time and reciprocal increase in gross energy intake per unit time.

During 1988, the advantage of a low cost seed source was notable for several females that had depleted their cone caches by mid-February. Much of their day was spent foraging far away from their depleted cache. Their body weights of 180-190 grams were well below the weights noted for females with substantial cone caches. When given seed additions, these squirrels made major weight gains of 40-50 grams in only a couple of weeks.

Within the same forest, the dominant conifers on a squirrel's territory seem to influence timing of reproduction as a result of handling time. Spruce (*Picea sp.*) seed has about the same caloric value as jack pine (Brink and Dean, 1964; Smith, 1968), but lacks serotinous bracts. Thus, handling time is less for a spruce cone than for a jack pine cone. Female squirrels with spruce cone caches tend to cycle before females with jack pine cone caches.

5.4.6 Food additions in other studies of tree squirrels

Sullivan & Sullivan (1982) found no change in timing of reproduction in Douglas squirrels (*Tamiasciurus douglasi*) when given extra food. Using the same methods with red squirrels, Sullivan (1990) found that food-addition resulted in a longer breeding season. However, only rough measures of timing (such as the percentage of scrotal males or lactating females trapped each month) were used. The actual mechanism behind the pattern was not explored, and monitoring was not individualized. Food-additions delivered in a "broad cast" fashion are unlikely to detect, or contribute to our understanding of the individual variation that shapes a species' breeding season because they often increase the density of animals in an area, and alter territorial systems (Klenner, 1990).

5.4.7 Conclusion

The asynchronous breeding season exhibited by red squirrels is caused by both variation in individual sensitivity to net energy gains in spring and variation in environmental conditions that determine these gains. When environmental conditions are similar, the timing and degree of net energy gain experienced by squirrels may differ as a result of variability in territory quality, handling time of food items, and strategies of foraging employed by different females.

5.5 Literature Cited

Baker, J. R. 1938. The evolution of breeding seasons. In: G. R. de Beer (ed.) *Evolution: Essays on Aspects of Evolutionary Biology*, Oxford University Press, London, 161-177.

Brink, C. B. and F. C. Dean. 1966. Spruce seed as a food of red squirrels and flying squirrels in interior Alaska. *J. Wild. Mgmt*. 30: 503-512.

Boutin, S. A. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems and the future. *Can. J. Zool*. 68: 203-220.

Bronson, F. H. 1989. *Mammalian Reproductive Biology*. University of Chicago Press, Chicago.

David, P. 1977. Sand Dune Occurrences of Canada. Report No. 74-230. Indian and Northern Affairs. National Parks Branch. Canada.

Gurnell, J. 1984. Home range, territoriality, caching behaviour and food supply of the red squirrel (*Tamiasciurus hudsonicus fremonti*) in a subalpine lodgepole pine forest. *Anim. Behav.* 32: 1191-1131.

Imms, R. A. 1990. The ecology and evolution of reproductive synchrony. *Trends in Ecology and Evolution* 5: 135-140.

Kenagy, G. J. 1980. Interrelation of endogenous annual rhythms of reproduction and hibernation in the golden-mantled ground squirrel. *J. Comp. Physiol.* 135: 333-39.

Kenagy, G. J. 1981. Endogenous annual rhythm of reproductive function in the non-hibernating desert ground-squirrel *Ammospermophilis leucurus*. *J. Comp. Physiol.* 142: 251-58.

Klenner, W. 1990. *The Effect of Food Abundance and Territorial Behaviour on Population Dynamics of the Red Squirrel.* Ph.D. thesis. University of British Columbia, Vancouver, B. C.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Millar, J. S. 1970. The breeding season and reproductive cycle of the western red squirrel. *Can. J. Zool.* 48: 471-473.

Millar, J. S. 1968. *The Reproductive Biology of the Western Red Squirrel.* Ph. D. thesis. University of British Columbia. Vancouver, B. C.

Modafferi, M. M. 1972. *Aspects of Reproductive Biology of the Red Squirrel (Tamiasciurus hudsonicus).* M.S. Thesis. University of Alaska. Fairbanks, Alaska.

Negus, N. C. and P. J. Berger. 1987. Mammalian reproductive physiology: adaptive responses to changing environments. In *Current Mammalogy,* H. H. Genoways (ed.) New York, Plenum Press. 149-173.

Price, K. (1989). Territorial Defence and Bequeathal by Red Squirrel Mothers in the Northern Boreal Forest. MS Thesis. University of British Columbia. Vancouver, B. C.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

Sanders, E. H., P. D. Gardner, P. J. Berger, and N. C. Negus. 1981. 6-Methoxybenzoxalinone: a plant derivative that stimulates reproduction in *Microtus montanus*. *Science* 214: 67-70.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels *Tamiasciurus*. *Ecol. Monogr.* 38(1): 31-63.

Smith, M. C. 1968. Red squirrel responses to spruce cone failure in interior Alaska. *J. Wildl. Mgmt.* 32(2): 305-317.

Sullivan, T. P. and D. S. Sullivan. 1982. Population dynamics and regulation of Douglas squirrel (*Tamiasciurus douglasii*) with supplemental food. *Oecologia* 53: 264-270.

Sullivan, T. P. 1990. Responses of red squirrel (*Tamiasciurus hudsonicus*) populations to supplemental food. *J. Mamm.* 71: 579-590.

Zirul, D. L. 1970. *Ecology of a northern population of the red squirrels, Tamiasciurus hudsonicus preblei (Howell).* M.S. Thesis. University of Alberta, Edmonton, Alberta, Canada.

Chapter 6

Variation in the probability of estrus and parturition in red squirrels

6.1 Introduction

It is probably rare in any population of wild animals for all reproductively capable adults to breed every season. North American red squirrels are no exception; the proportion of females breeding from year to year can vary by as much as 50% (Smith, 1968).

Two hypotheses for variable fecundity in red squirrels have been made by population ecologists. The "over-winter energy" hypothesis states that the larger the cone crop size, the greater squirrel productivity will be in the following spring (Millar 1970, Searing 1975). Empirical support for this idea has been borne out in studies by Rusch and Reeder (1978), Millar (1970), and Searing(1975).

Smith (1968), Kemp and Keith (1970) and Kelly (1978) obtained results that conflicted with the "over-winter energy" hypothesis. In years following small cone crops, but preceeding large crops, they found increased fecundity in red squirrels. Kemp and Keith (1970) posed the "anticipation" hypothesis to explain this pattern. They proposed that "nutritionally superior" flower buds stimulate squirrel reproduction in advance of a high cone crop.

Neither of these hypotheses addresses changing levels of primiparity, which is the major source of the variation in the proportion of females breeding in a given year. The proportion of yearlings breeding from one year to another can differ by as much as a factor of four (Rusch & Reeder, 1978). As has been found for some ground squirrel species

(Heaney, 1981), female red squirrels may delay breeding until they are two or three years old.

Bronson (1985) suggested that annual patterns of breeding reflect a relationship between the caloric costs of a female's reproductive effort and the costs and gains associated with foraging. The proportions of first-time breeders in red squirrel populations increase when food is abundant and when spring temperatures are mild (Chapter 5), suggesting that net energy for reproduction varies more for young squirrels than for older multiparous females (Kemp and Keith, 1970; Rusch & Reeder, 1978).

This study attempts to identify potential intrinsic and extrinsic factors that influence the probability of estrus, parturition and primiparity in red squirrels. It is proposed that the variation in annual primiparity exhibited by red squirrels is caused by variation in net energy gain to individuals during the breeding season. To test this hypothesis, the probability of estrus and parturition was compared in control and treatment groups of squirrels of known age or of known reproductive status. Individuals in the treatment groups were given super-abundant pulses of food (sunflower seeds) prior to the breeding season. Several predictions were:

1. The probability of estrus, pregnancy and primiparity in treatment groups will exceed that of control groups.
2. Within the control groups, primiparity should be greatest when net energy gains are highest (i.e. following a good cone crop and a mild winter and spring)

Two hypotheses for age-related constraints on net energy gain in the spring are:

1. Young animals are more limited by food resources than adults because they are still growing: growing squirrels use energy for growth rather than reproduction.
2. Juveniles are less efficient than adults in the extraction and consumption of jack pine seeds.

Because red squirrels are resource defenders, variation in territory quality could also influence costs of foraging, and as a consequence, the ability to reproduce. It has been suggested that with the exception of peak cone crop years, many red squirrel territories fail to supply enough food to meet a squirrel's metabolic costs of estrus and gestation (Kemp and Keith, 1970; Rusch & Reeder, 1978; Klenner, 1990). The distribution, abundance and quality of food trees near the midden, and the size of territory defended should influence reproduction in red squirrels. The following predictions address this hypothesis:

1. Sequential breeders (producing a litter for two years in a row) will have more pine trees near the cache than females that fail to produce a litter for two years in a row.
2. Sequential breeders will have larger territories than sequentially non-breeding female adults.

Wauters & Dhondt (1989) found that European red squirrels (*Sciurus vulgaris)* weighing less than 300 g had less than a 5% probability of coming into estrus. Food shortage and intraspecific competition prevented some squirrels from attaining the energy required for ovulation and gestation to term. Like the European red squirrel, the probability of estrus and parturition in American red squirrels may depend on the attainment or maintenance of a critical energy balance (Bronson and Manning, 1991).

6.2 Methods

6.2.1 Study Site

Research was conducted over a three year period (1988-90) in the Athabasca River Sandhills southwest of the village of Fort Assiniboine in central Alberta (54°17' N, 114°50' W). The study site consists of fragmented islands and peninsulas of jack pine (*Pinus banksiana*) stands on top of ancient sand dune formations (David, 1977). Black spruce (*Picea mariana*), white spruce (*Picea glauca*) and larches (*Larix laricina*) are present in small patches in lowland areas.

Squirrels were trapped at their middens, mounds of conifer bracts containing food caches and underground nesting tunnels associated with each territory. About 60 middens were located in conifer forest fragments adjacent to a 10 km county road bisecting the study area. The road was cleared in winter allowing access to middens during periods of deep snow. Another 200 active squirrel middens were located within the boundries of two study grids (approx. 1 km^2 each) maintained by K. Larsen (Univ. of Alberta).

6.2.2 The squirrel population

Densities of two to three squirrels per hectare were typical in the jack pine stands. The territory sizes of individual squirrels ranged from 0.2 to 1.2 ha. (Larsen, pers. comm.) and were defended by vocalization, scent-marking and physical aggression (pers. obs.).

Each fall, three age-reproductive classes of female squirrels: multiparous, nulliparous, and juvenile, were easily determined by nipple condition (Layne,1954) and body size. Juveniles (females less than one year old) were known from birth records during the 1990 breeding season, and by low fall weights and appearance (Kemp and Keith 1970) in the two other breeding seasons. Several females were nulliparous for 3 years, indicating that factors associated with either the female or the environment can delay breeding for a relatively long time. Squirrels producing a litter for the first time were referred to as primiparous females. Females producing a litter in the spring following their birth were called primiparous juveniles, whereas older first time breeders were referred to as primiparous adults.

6.2.3 Additions of sunflower seeds

During the breeding seasons of 1988, 1989 and 1990, the proportion of adult females exhibiting estrus and producing litters were monitored. A subset of these females received an unlimited cache of sunflower seeds prior to and during the breeding season. A plastic bucket filled with sunflower seeds (8 kg) was placed on a tree, or buried in the midden on each squirrel's territory. The buckets were checked every week and were kept full.

Thirteen squirrels received seed additions throughout the winter and spring of 1988-1989, while seventeen (nine in 1988 and eight in 1989) received them just prior to the breeding season in spring. Winter food-additions were continuous on each treatment territory from October 31 until the parturition of each female. Spring food additions were made on February 14 in both years, and were maintained through parturition of each treatment female.

During the preceding winter and throughout the spring of 1990, twelve juvenile squirrels were given sunflower seeds. Over-winter survival, growth and fecundity of these juveniles was compared with the same variables in juveniles and nulliparous adults that were feeding on a normal diet of conifer seeds.

To determine if food availability in the fall affected over-winter survival and ability to breed the following spring, sunflower seeds were provided to eight nulliparous females during October 15 to December 15, 1989.

6.2.4 Trapping and handling of squirrels

Details of the trapping schedule are given in Section 5.2.4. Tomahawk live traps baited with peanut butter were used to catch squirrels. From the trap, the squirrel was transferred to a cone-shaped cloth bag, in which the ear tag number, weight (g), zygomatic width (mm) and reproductive status were determined. The mean spring weight of each female was calculated from four or more measurements made during late February and March. Zygomatic widths were used to calculate growth rates and to provide an assessment of body sizes independent of weights. Zygomatic width is correlated with cranial width, whole body length and hindfoot length and is thus a reasonable measure of body size in red squirrels (see Appendix III).

Vaginal smears, vulva swelling and mating chases were used as indicators of dates of proestrus and estrus (Modafferi, 1972). When the date of estrus had been determined, the female was left alone until her expected date of parturition, at which point trapping was resumed. Sudden loss of weight and lactation signaled births. If the location of the squirrel's nest was not obvious, the lactating female was radio-collared and tracked to the nest to ascertain the successful production of a litter.

6.2.5 Chi-square and logistic regression analysis

The probabilities of reproductive events were modeled by logistic regression. (Hosmer & Lemeshow, 1989). Albon *et al.* (1983) demonstrated that the relationship between the probability of being fertile and body weight in red deer (*Cervus elaphus*) can be fit by a logistic curve. Wauters and Dhondt (1989) used logistic regressions to determine variables influencing the fertility of European red squirrels in two habitats. The specific form of the logistic regression model used by the above workers and in this study is:

$$P\,(Y_i = 1) = \frac{\exp G(x_i)}{1 + \exp G(x_i)}$$

where $P(Y_i = 1)$ refers to the probability of estrus or parturition being equal to 1.0, and where $G(x_i)$ includes the y-intercept and regression coefficients associated with a series of independent variables: age-reproductive class (juvenile=1, nulliparous=2, multiparous=3), fall weight, mean spring weight, spring gain (weight gain after winter low), body size (zygomatic width), food level (control=0, or treatment=1) and two measures of body fat. An individual body fat index (BFI) was derived by dividing mean spring weight by zygomatic width. Another index of relative body fat (FAT) was calculated by subtracting the mean spring weight calculated for each individual from a predicted value of weight based on the whole female population. The predicted value was derived from a regression of mean spring weights (y) on zygomatic widths (x): Y(grams) = 15.8 x(mm) - 238.4 ; $r^2 = 0.60$, $p < 0.0005$, N=108). Multivariate models were calculated by stepwise logistic regression with p-value < 0.05 set as the screening criterion for selection of candidate variables (Hosmer & Lemeshow, 1989).

6.2.6 Food and foraging behavior

Food items consumed by squirrels, their handling times, and distances that squirrels were found away from the midden were recorded whenever squirrels were located and recognized by colored ear tags. Two hundred hours of focal animal sampling (Altmann, 1974) were completed to describe feeding and foraging behaviour of the female squirrels.

6.2.7 Territory quality assessment

To test the hypothesis about territory quality, several assumptions were made. First, bioenergetic costs of foraging depend on the distribution and abundance of trees on a squirrel's territory. When hoarding cones at a midden, squirrels act as central-place foragers, harvesting cones at a distance and carrying them back to a storage place. Field tests of the central place foraging models proposed by Orians & Pearson (1979) and Schoener (1979) report that red squirrels balance the profitability of cones with distances traveled (Elliot, 1988): long travel distances correlated with lower cone handling times. If squirrels optimize their foraging behaviour in response to distances traveled, then the location of middens should minimize travel costs to obtain cones and at the same time maximize cone availability. Thus, in terms of this concept, dense patches of large mature conifers near the central place forager's midden indicate a higher quality territory than a midden surrounded by a sparse mixture of deciduous trees and conifers.

Density, sizes, and species composition of trees located within a 20 m radius of middens defended by adult females with different breeding histories were compared. Thirty-one territories met the criteria that the same adult (older than 1 year) female had been resident for two years. Fourteen of the middens belonged to females that had litters in 1988 and 1989, while seventeen middens belonged to adults that did not give birth during the same period.

The quality of the forest around each midden was estimated in four quadrats placed north, south, east and west within 20 m of the midden. Each quadrat was 50 m^2. The jack pine trees in each quadrat were counted, and the mean density of jack pine per midden site was calculated by averaging the four counts from the quadrats. The DBH (diameter at breast height) was measured for every jack pine in each quadrat. The percentage of jack pine and deciduous trees (aspen and birch) in each quadrat was also recorded. Mean DBH and mean percentages of pine and deciduous trees were calculated from the quadrat measurements. It was predicted that the breeding females would have higher densities of large DBH pines and lower densities of deciduous trees than non-breeding females. Data on territory quality were collected by assistants with no knowledge of the reproductive status of the female associated with the midden.

6.3 Results

The data presented below came from squirrels that failed to reproduce and monoestrus squirrels that successfully produced a litter. During the three year study of over 100 females, only one case of a multiple estrous cycle without conception was noted. There were two cases of multiple litters. In one, a multiparous female with food supplements produced two litters, one at the end of March and another in June. Another multiparous control female lost nestlings in May and produced a second litter relatively late in the season (mid-July). These postpartum estrus cycles occurred while the female was still lactating.

6.3.1 Factors influencing the probability of estrus and parturition

The logistic model from data for all females (all ages and food levels) suggested that except for zygomatic width and weight gain from winter to spring, all the variables considered singly were significant factors in predicting the probability of estrus in female squirrels (Table VI-1). However, the model selected by a stepwise procedure included only age-reproductive class and food level (TableVI-1). In the equation of the final model for the probability of estrus $G(x_i) = -2.01 + 1.82$ age-rep $+ 2.51$ food level.

Logistic regression suggested that when considered singly, all the independent variables were significant factors in predicting the ability to produce young (Table VI-2). The stepwise procedure selected age-reproductive class, food level, and mean spring body weight for inclusion in a multivariate predictive model (Table VI-2). In the equation for the final model $G(x_i) = -14.76 + 1.19$ age-rep $+ 0.061$ mean spring weight $+ 1.75$ food level.

Table VI-1. Logistic regression model predicting the probability of estrus in female red squirrels (N=115). Significance levels: * = P<.05; ** = P<.01; *** = P<.001

Adjusted Chi-Square of variables not in the model (d.f.=1)

Variable	Chi-Square	P
Age-reproductive class	10.67	0.0011
Body fat index (BFI)	10.21	0.0014
Fall body weight	9.72	0.0018
Fat (relative)	9.00	0.0027
Spring body weight	8.85	0.0029
Food level	3.9	0.0480
Spring gain in weight	1.75	0.1850
Body size (zygomatic width)	1.23	0.2683

Model	Age-rep	Food level	Body fat	χ^2 model	d.f.	P
A	8.99**			10.4	1	.001
B	10.68***	6.46**	N. S.	18.3	2	.001

Final model selected = model B

6.3.2 Experimental effects of food on patterns of survival, estrus, primiparity and parturition

In 1990, over-winter survival was significantly higher for juveniles and nulliparous females given sunflower seeds than for females of the same age and reproductive class in the control groups ($Z_{(2)} = 4.2$, $P < 0.05$). The proportion and number of animals surviving from October 1989 until July 1990 is given under the "survival" heading in Table VI-3.

In all years, winter and spring food additions increased the proportions of juveniles and nulliparous females having an estrus and a litter (Table VI-3). In 1990, the percentage of juveniles having a litter was increased from 14% in the control group to 80% in response to the sunflower seeds. Seed additions in winter and spring caused a ten-fold increase in primiparity in 1989. The short term pulse of extra food in the fall of 1989 did not increase the occurrence of puberty (first estrus) or primiparity (first successful pregnancy) during the breeding season of 1990.

Multiparous females showed no increase in the probability of having an estrus or a litter in response to the seed-additions (Logistic regression, $p > 0.5$). All of the multiparous females that were given seed additions bred and produced litters (N=15). Only three out of 46 multiparous females in the control groups failed to produce litters during the three breeding seasons.

Table VI-2. Logistic regression model predicting the probability of producing a litter in female red squirrels (N=115).
Significance levels: * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$

Adjusted Chi-Square of variables not in the model (d.f.=1)

Variable	Chi-Square	P
Age-reproductive class	21.06	0.0001
Body fat index (BFI)	22.89	0.0001
Fall body weight	20.42	0.0001
Fat (relative)	12.13	0.0005
Spring body weight	25.29	0.0001
Food level	5.11	0.0238
Spring gain in weight	5.50	0.0191
Body size (zygomatic width)	12.89	0.0003

Model	Mean sp wt.	Food level	Age-rep	χ^2 model	d.f.	P
A	18.91***			29.3	1	0.001
B	18.47***	4.53*		34.35	2	0.001
C	25.29***	4.78*	6.85**	41.34	3	0.001

Final model selected= model C

6.3.3 Annual variation in productivity and primiparity

In squirrels on normal diets (controls), the percentage of females producing litters differed annually (Table VI-3). In 1988 and 1989, the percentage of parous females did not differ despite the contrasting size of cone crops and climatic conditions (Chapter 5, Section 5.2.3). In 1990, the total proportion of females having young was larger by 25% than in two previous years (Tukey multiple comparisons test, $P < 0.05$). This difference was caused by an increase in the proportion of first time breeders in 1990 (Table VI-3). Almost half of the litters came from primiparous females in 1990, while in 1988 and 1989 only 22% and 15% of the litters (respectively) were produced by first time breeders (Chapter 5, Section 5.2.3).

During the three year study, the estimated percentage of multiparous females producing a litter ranged from 83% to 100%, whereas the range in nulliparous adults was 7% to 64 % (Table VI-3). The average percentage of multiparous females failing to produce a litter was only 9 %. In contrast, the average proportion of nulliparous adults failing to produce litters over the three seasons was 70%.

Of the three years, 1990 had the best bioenergetic conditions for reproduction: cone production was relatively high in the fall preceding the breeding season, while temperatures during March were above average. The breeding seasons of 1988 and 1989 were relatively poor in terms of bioenergetic conditions. Either food availability was low, or ambient temperatures in winter and spring were lower than average (Chapter 5, Section 5.2.3). The breeding season of 1988 followed a poor cone crop. Despite a very mild spring, squirrels relied extensively on buds and cones that remained on trees. The season of 1989 followed a fairly heavy cone crop, but mean temperatures in February and March were colder than the 10 year averages (Chapter 5, Section 5.2.3).

Table VI-3: Effects of sunflower seed additions on life history traits of female squirrels. (Numbers of primiparous females recorded on control grids by K. Larsen are included for each season.)

a. 1988 Breeding Season *

Female class & treatment	Survival	Estrus	Parturition
Nulliparae Control (N=6)	NA	0.16	0
Nulliparae Seed-add (N=4)	NA	1.00	0.75
Multiparae Control (N=6)	NA	1.00	1.00
Multparae Seed-add (N=5)	NA	1.00	1.00
All Female Controls (N=12)		**0.58**	**0.50**

Number of primiparous adult females on grids = 6/29
* Females given seed additions had a higher probability of having an estrus and a litter than controls, Fisher Exact, p=.002

b. 1989 breeding season

Female class & treatment	Survival	Estrus	Parturition
Nulliparae Control (N=14)	NA	0.57	0.07
Nulliparae Seed-add (N=11)	NA	1.00**	.70**
Multiparae Control (N=11)	NA	1.00	0.91
Multiparae Seed-add (N=10)	NA	1.00	1.00
All Female Controls (N=25)		**0.72**	**0.44**

Number of primiparous adult females on grids = 4/26
** G-test, P <.05, Seed addition differs from the proportions in matched control group.

c. 1990 Breeding season

Female class & treatment	Survival	Estrus	Parturition
Juveniles Control (N= 21)	0.66 (14)	0.14	0.14
Juveniles Seed-add (N=12)	0.83 (10)	0.95**	0.80**
Nulliparae Control (N=30)	0.83 (25)	0.84	.064
Nulliparae Fall seed add (N=8)	1.00 (8)	0.75	0.50
Multiparae Control (N=28)	0.82 (24)	0.96	0.83
All Female Controls (N=79)	**0.79**	**0.73**	**0.60** †

Number of primiparous adult females on grids = 18/43
** G-test, P <.05, Seed addition differs from the proportions in matched control group.
† No juveniles were known to breed in the previous two seasons. When juveniles are not included, the proportion of adults breeding = 0.73.

6.3.4 Factors influencing puberty

To determine what factors affect the ability of a female to attain puberty (first case of estrus), logistic regressions were repeated on the same data set described earlier, but with multiparous females and seed-addition animals excluded. When all of the remaining independent variables were considered together none were significant in predicting the probability of estrus in squirrels that had no breeding experience. This result is assumed to have occurred because of too many missing values (45 squirrels were excluded due to missing data).

The same data set was re-examined with mean spring body weight and age as the only variables in a logistic model predicting the probability of puberty. This analysis was more reliable because only two of 64 cases were missing mean spring weight values. Only mean spring weight was significant (Wald Chi-Square= 4.5, $p=0.036$). In a single factor logistic regression $G(x_i) = -10.39 + 0.053$ mean spring weight (g) (Chi-Square = 8.37, d. f. =1, $p = 0.004$). Figure VI-1 illustrates the curve generated by this logistic model and shows that higher weights increase the probability of attaining puberty in both juvenile and adult nulliparous female red squirrels. In the population as a whole, a nulliparous female with a mean spring weight of 190 grams has an estimated probability of 0.4 of coming into estrus, while a female weighing 220 grams has a probability of 0.8.

6.3.5 Factors influencing primiparity (production of first litter)

In another logistic regression including only nulliparous females from the control group, all variables except spring gain and age class were significant when considered singly as predictors for probability of primiparity (Table VI-5). Mean spring weight was the only variable selected by the stepwise procedure for predicting the probability of primiparity in squirrels. In the logistic equation, $G(x_i) = -15.7 + 0.074$ mean spring weight (g).

The probability of producing a litter for the first time was below 5% when mean spring weight was 180 grams. When food level is reconsidered for nulliparous females (using the equation on page 110), seed addition improved the probability of primiparity (Figure VI-2). For example, a 200 g female on a normal diet had a 20% probability of producing a litter, while a female of the same weight on a sunflower seed diet had a 70% probability of doing the same.

Multiparous females ranged in weight from 195-252 grams, well-above the estimated 180 g mean spring weight threshold for parturition. Despite this range in weights there was no significant variation in the probability of estrus or parturition in multiparous females as a function of weight.

6.3.6 Body growth patterns

From October to April 1989-90, increases in zygomatic widths were greater in juvenile squirrels than in nulliparous and multiparous adults. Zygomatic widths of juveniles grew, on average, 0.8 mm (Paired t-test = -6.76, df=16, p=0.002). Nulliparous females showed a small increment in zygomatic width: 0.3 mm (Paired t-test = -5.08, df=28, p=0.003). Multiparous females showed no significant growth or change in the width of their cheek bones. Zygomatic growth in juveniles was not increased by additional food (repeated measures ANOVA, F=0.29, P=0.5972).

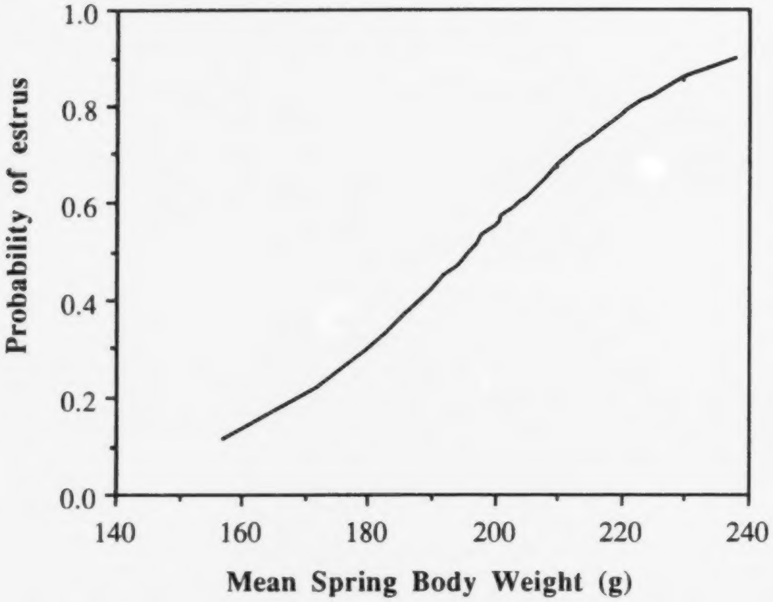

Figure VI-1. Logistic curve representing the probability of estrus in juvenile and nulliparous red squirrels.

Table VI-4. Logistic regression model predicting the probability of primiparity in red squirrels (N=42).

Significance level: * = P< 0.05; ** = P< 0.01; *** = P< 0.001

Adjusted Chi-Square of variables not in the model (d.f.=1)

Variable	Chi-Square	P
Age class (juvenile or not)	0.98	0.3216
Body fat index (BFI)	7.46	0.0063
Fall body weight	4.51	0.0338
Fat (relative)	4.64	0.0312
Spring body weight	8.52	0.0035
Spring gain in weight	0.24	0.6203
Body size (zygomatic width)	4.32	0.0376

Model	Mean spring body weight	$\chi2$ model	d.f.	P
A	8.52**	9.8	1	**

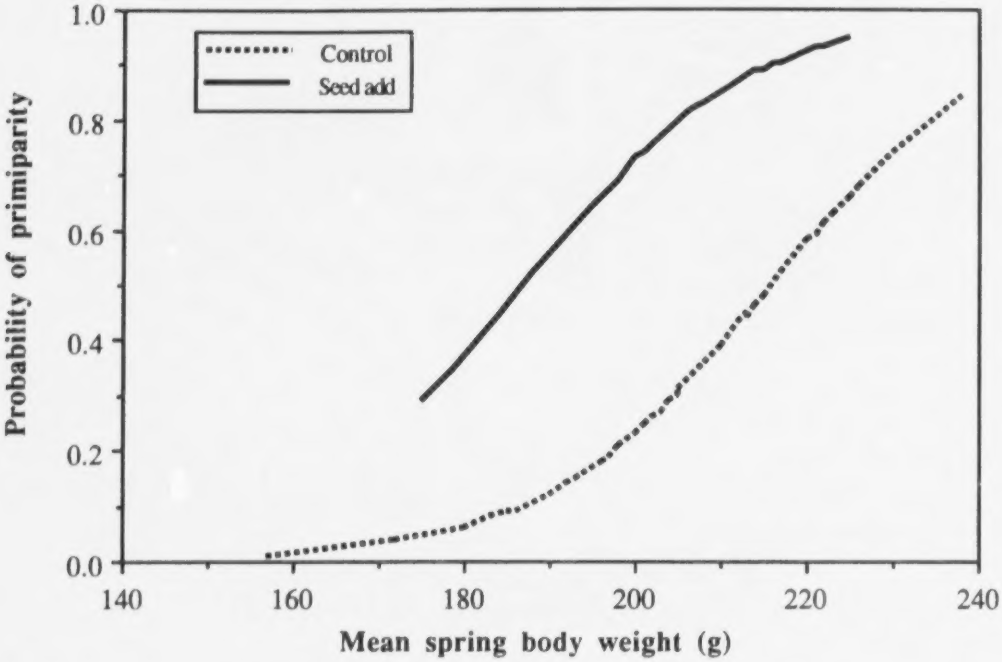

Figure VI-2. Logistic curves representing the probability of primiparity as a function of diet (sunflower seed versus jack pine seed (control)).

6.3.7 Feeding efficiencies

In a small, controlled feeding study conducted in September of 1989, age and zygomatic width were related to the feeding time required to process a pine cone. Juvenile squirrels with small zygomatic widths (n=6) required more time than larger adult females (N=6) to extract and consume the seeds from serotinous jack pine cones (Figure VI-3).

Data collected during trapping sessions and focal animal studies (October-December, 1989) indicated that juvenile squirrels (N=27) took about 2.6 minutes longer than adults (N=99) to extract seeds from a single cone (t = -4.34, p = 0.001). Juveniles spent a mean time of 424 ± 288 seconds to handle and ingest the seeds from a single jack pine cone, while adults averaged 268 ± 113 seconds to do the same. All records were from continuous feeding bouts on whole cones, uninterrupted by vigilance or repositioning.

6.3.7.1 Preliminary data on cone-stripping behavior

One behaviour that was noted in many adult squirrels (45 cases in 135 hours of observation on 18 different adults), but not in juvenile squirrels (0 cases in 60 hours of observation on 8 different juveniles), was bract stripping. The basal one third of a jack pine cone consists of thickened bracts, and a squirrel must remove these bracts before it can begin to extract seeds from the cone. Adult squirrels were observed stripping bracts from the basal region of the cones in the fall. After stripping the cones, they buried them in piles (two to 20 cones) in small depressions on the surface of the midden. The piles of stripped cones were usually covered with 15-40 unstripped cones. Small piles of six to 20 stripped cones were sometimes stored at the bases of trees on the territory (pers. obs.).

In a survey conducted in mid-October of 1989, stripped cones accounted for as much as 40% of the cones cached in piles on the top of some middens. All of the middens with stripped cones belonged to adult squirrels (N=50). Freshly stripped cones were not found at any of the middens occupied by juveniles (N=35). On average, "stripping" required two minutes (34 timed observations of adult squirrels) and accounted for 50% of the mean handling time associated with extracting the typical 0.14 g of seed from a jack pine cone.

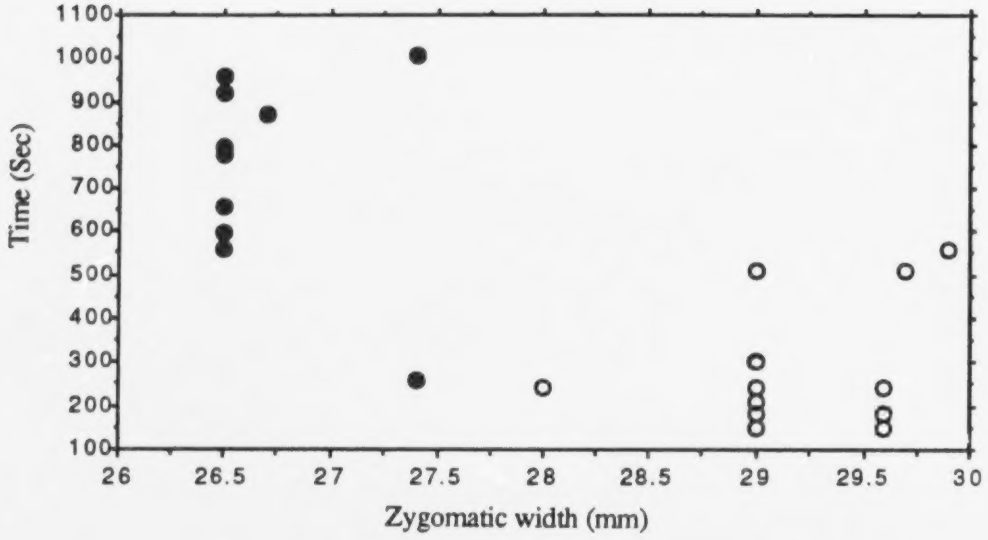

Figure VI-3: Time required to extract the seeds from one jack pine cone as a function of zygomatic width and age. Squirrels with small zygomatic widths (< 27.9 mm) were juveniles, (N=6), while the others are adults, (N=6).

6.3.8 Interrelationships between breeding and "territory quality"

Territory quality near the midden differed between adult females that produced litters for two consecutive breeding seasons (sequentially multiparous) and those that delayed or failed to breed during that period (sequentially nulliparous). In the 200 m^2 forest samples, sequentially multiparous females had an average of 44 trees from which to harvest and feed, while the nulliparous females had a mean of 24 trees ($t = 3.5$, d.f. = 29, $p = 0.0015$). Contrary to the original predictions, the sites belonging to nulliparous females had trees with significantly larger diameters at breast height (DBH) than those associated with sequentially multiparous females ($t = 3.5$, d.f.= 29, $p = 0.005$). DBH's were negatively correlated with the density of pine trees, and as shown in Figure VI-4, the majority of the females that failed to breed had middens near relatively large trees growing at low densities.

The opposite pattern was noted for most sequential breeders. They had middens in relatively dense stands of medium sized pine trees.

The mean percentage of deciduous trees near the middens of sequentially nulliparous females (16%) was higher than the value for the sequentially multiparous females (6.6%). However, the difference was not statistically significant ($t = 1.56$, d.f.=29, $p = 0.129$).

Contrary to original prediciton, territory size did not differ between the sequential breeders and non-breeding squirrels (t-test, d.f.=29, $p = 0.20$).

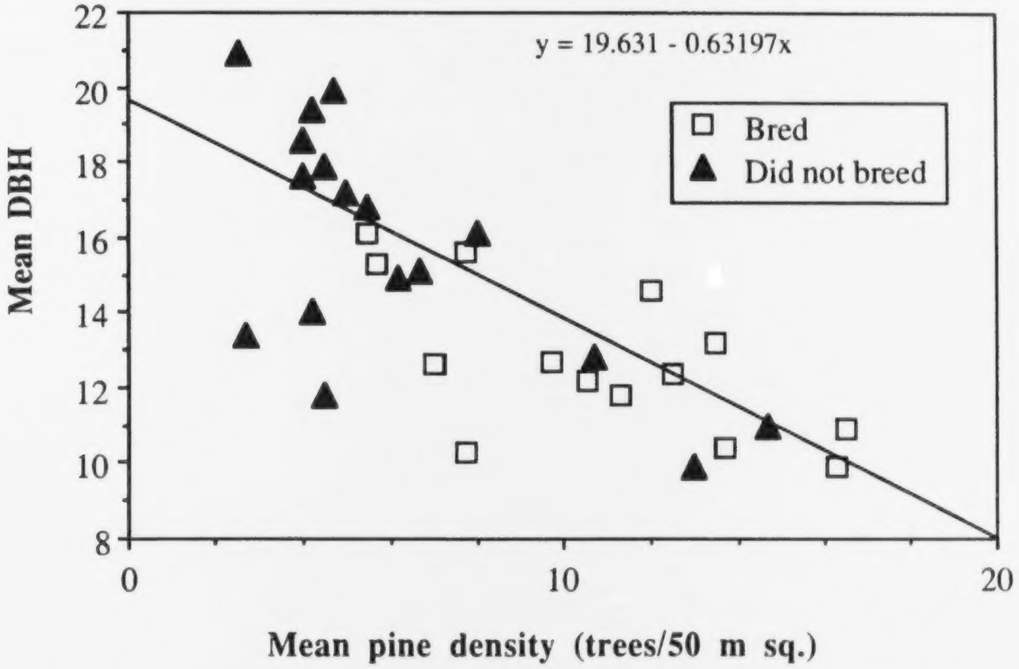

Figure VI-4. Mean density and DBH (cm) of jackpines on territories of red squirrels ($r^2 = 0.47$). Females that produced litters for two consecutive years (Bred) had middens in areas of high pine density with smaller DBHs. Adult females that did not breed for two consecutive years had middens in areas with a low density of large DBH pines.

6.4 Discussion

In populations of mammals, variable access to food resources has been shown to have lasting consequences for individual reproductive success, both in theory and in real life histories (Clutton-Brock, 1988). Although there are interesting exceptions, unrestricted food intake enhances growth rates, advances age of puberty, increases litter size, and improves other life history patterns of animals (Boutin, 1990). Food has been considered a limiting factor whenever its experimental addition results in an increase in performance in a species (Boutin, 1990). The results of seed additions in this study suggest that squirrels become less food-limited as they get older. The probability of estrus and conception was most improved by seed additions to juvenile and nulliparous squirrels. In contrast, no significant increase in the probability of having an estrus or a litter resulted from the seed additions given to multiparous females.

The proportion of multiparous females breeding each year was consistently high (above 80%), while the proportion of juveniles breeding was consistently low (below 15%). The source of greatest variation in both the probability of estrus and parturition was found in nulliparous squirrels known to be older than 20 months at the start of a breeding season. These results suggest that under normal conditions juveniles and some nulliparous adults either experience lower net energy intake than older and multiparous females or that they have higher metabolic costs.

6.4.1 Relationship between cone crops and climate and primiparity in red squirrels

Patterns of primiparity suggest that either a small cone crop or extremely low temperatures in spring limit reproduction in red squirrels, while a large cone crop coupled with mild weather "permits" more females to breed. These results, and the responses to additional food, support the contention that primiparity in squirrels is limited mainly by net energy gain in the spring.

6.4.2 Age effects on net energy gain

For at least two reasons, age plays an important role in determining the amount of energy available for reproduction in squirrels. First, because juveniles and nulliparous females are still growing at the time of the breeding season, energy is obviously allocated to growth of somatic tissue. Thus, under conditions in which food is costly to obtain, energy may not be obtained quickly enough to support both reproduction and growth. Allocation of energy to growth seems to take precedence over allocation to reproduction, but only to a limited extent. Additional food did not change growth or weight trajectories in juveniles, but caused large differences in fecundity. The rate of growth therefore seems to be a relatively inflexible aspect of red squirrel life history. Under normal conditions within a territory, food may be adequate to meet costs of somatic growth in juvenile and yearling squirrels, but rarely adequate for reproduction as well.

A second reason that age influences the amount of energy available to female squirrels may involve the ability to process food items. Because juveniles require more time to extract energy from cones than adults, they may fail to obtain enough energy per unit of time to cover the costs of reproduction. Measurements of handling time were made in September to December, when juveniles were both relatively small and inexperienced with cones, so one cannot be certain that handling time is an important factor in the breeding season, after squirrels have spent a winter handling cones and have grown. In other studies, juvenile red squirrels handled food items more optimally when they were able to watch an adult squirrel than when they were reared without a role model (Weigl & Hanson, 1980). Thus, experience and learning is probably required to optimize handling time and shift net energy gain enough to support costs of reproduction.

Field observations suggest that as squirrels get older, they alter the long-term costs of handling time by stripping cones. Stripping cones in the fall would theoretically save energy in the winter when metabolic costs often quadruple (Pauls, 1981). Cone stripping appears to be a learned behaviour that appears after the juvenile year. To my knowledge, cone stripping has not been observed in other red squirrels studies. Grey squirrels remove the husks of walnuts before burying the nut (Jacobs, 1989)

6.4.3 Influence of territory quality

For nulliparous adult females, factors other than age and experience are needed to explain the differences in reproduction. It is unlikely that nulliparous females (age 2-3 yrs) are limited by their physical ability to process cones. Comparisons of "territory quality" suggest that energy for reproduction may be limited by the structure of the forested area around the midden defended and foraged upon by the squirrels. The density of pine trees was relatively high near the middens of the sequentially successful breeders, but it was low near those females that delayed or failed to produce young. These results suggest that a high density of trees of moderate size near a midden may provide better bioenergetic conditions for a squirrel than a site having fewer but larger trees. It is logical to predict that silvicultural spacing affects metabolic costs of foraging, but this remains to be verified.

Altered foraging and cone handling behaviour, combined with the completion of growth, may allow females with low conifer tree densities near the midden to eventually breed. Otherwise, one would expect that sequentially nulliparous females would delay breeding for their entire lives, or would compete for access to "better" territories. Contrary to the latter prediction, site tenacity in red squirrels has been shown to be quite strong.

Removals done by K. Larsen (pers. comm.) at the same study site showed that non-breeding females on established territories did not move to "better" territories. However, in studies where squirrels were removed from territories adjacent to a study animal, territory holders "held on" to their properties and expanded them (Price,*et al.*, 1986). It seems likely that red squirrels may combine learning and expansion of territorial ownership to optimize foraging.

In a preliminary study at the Athabasca Sandhill's site, Boonstra (pers. comm.) found differences between nulliparous and multiparous red squirrels in aspects of foraging behavior. During the cone-harvesting period in late August and September, nulliparous females were more likely to harvest cones near the midden (< 40 m), whereas multiparous females focused on harvesting cones away from the midden (> 40m). Although the former pattern associated with nulliparous females is optimal during the fall, it would theoretically result in higher foraging costs during the spring, once the winter cache was depleted. Cones would be less available near the midden, forcing the squirrel to travel more to acquire seeds. Squirrels probably learn to modify their foraging and handling behavior to optimize net energy gain in the spring. Further studies of the ontogeny of foraging behaviour in this species should be particularly rewarding.

6.4.4 Body condition and reproduction

Recent studies suggest that ovulation does not depend on critical levels of body fat, but on whole-body energy balances (Bronson and Manning, 1991). Thus body fat levels (increases in body weight) may be viewed as a side effect of a positive energy balance rather than a causal mechanism of reproductive events.

Like the European red squirrel, the American red squirrel appears to have weight thresholds for estrus and parturition. Both species experience similar extrinsic factors such as periodicity of mast crops and climate. Still, whole-body energy balance is controlled differently in the two species by their contrasting social systems. In the larger European species, social rank determines access to food-rich patches, and consequently influences reproductive success. As food supply dwindles in spring, all European red squirrels share a reduction in food availability (Wauters and Dhondt, 1989). The same positive covariation exists between age, weight and breeding in the smaller, North American red squirrel, but in absence of a social ranking system.

In the American red squirrel, territory quality and age-related foraging behavior influences rates of energy intake, body weight and fecundity far more than intraspecific competition in the spring. Once a territory has been acquired, the North American red squirrel in a jack pine forest has fairly consistent control over its food supplies. Those individuals with high net energy gains in the spring have a higher probability of producing a litter than squirrels with low net energy gains. Improvements in efficiency of foraging, handling and caching, rather than direct aggression with conspecifics becomes critical for attaining the positive energy values required for reproduction and associated with high weights in American red squirrels.

6.5 Literature Cited

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior* 49: 227-267.

Albon, S. D., B. Mitchell and B. W. Staines. 1983. Fertility and body weight in female red deer: a density-dependent relationship. *J. Anim. Ecol.* 52: 969-980.

Albon, S. D., B. Mitchell, B. J. Huby and D. Brown. 1986. Fertility in female red deer (*Cervus elaphus*): the effects of body composition, age and reproductive status. *J. Zool. Lond.* 209: 447-460.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems and the future. *Can. J. Zool.* 68: 203-220.

Bronson, F. H. 1985. Mammalian reproduction: an ecological perspective. *Biol. Reprod.* 32: 1-26.

Bronson, F. H. and J. M. Manning. 1991. The energetic regulation of ovulation: a realistic role for body fat. *Biol. Reprod.* 44: 945-950.

Clutton-Brock, T. H (ed.) 1988. *Reproductive Success*. University of Chicago Press. Chicago.

David, P. 1977. Sand Dune Occurences of Canada. Report No. 74-230. Indian and Northern Affairs. National Parks Branch. Canada.

Elliot, P. F. 1988. Foraging behavior of a central-place forager: field tests of theoretical predictions. *American Naturalist* 131(2): 159-174.

Gurnell, J. 1984. Home range, territoriality, caching behaviour and food supply of the red squirrel (*Tamiasciurus hudsonicus fremonti*) in a subalpine lodgepole pine forest. *Anim. Behav.* 32: 1191-1131.

Hosmer, D. W. and S. Lemeshow. 1989. *Applied Logistic Regression*. John Wiley & Sons. New York.

Jacobs, L. 1989. The cache economy of the gray squirrel. *Natural History* 10:41-46.

Kelly, D. G. 1978. *Population Density, Territoriality and Foraging Ecology of Red Squirrels (Tamiasciurus hudsonicus) in Black and White Spruce Forests of Interior Alaska.* M.S. Thesis. University of Alaska, Fairbanks, Alaska.

Kemp, G. A. and L. B. Keith. 1970. Dynamics and regulation of red squirrel (*Tamiasciurus hudsonicus*) populations. *Ecology* 51(5): 763-779.

Klenner, W. 1990. *The Effect of Food Abundance and Territorial Behaviour on Population Dynamics of the Red Squirrel.* Ph.D. Thesis. University of British Columbia, Vancouver, B. C.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Millar, J. S. 1970. Variations in fecundity of the red squirrel, *Tamiasciurus hudsonicus* (Erxleben). *Can. J. Zool.* 48: 1055-1058.

Modafferi, M. M. 1972. *Aspects of Reproductive Biology of the Red Squirrel (Tamiasciurus hudsonicus).* M.S. Thesis. University of Alaska. Fairbanks, Alaska.

Orians, G. H. and N. E. Pearson. 1979. On the theory of central place foraging. In: *Analysis of Ecological Systems.* D. H. Horn, R. Mitchell and G. R. Stairs (eds.). Ohio State University Press. Columbus. p. 155-177.

Pauls, R. W. 1981. Energetics of the red squirrel: a laboratory study of the effects of temperature, seasonal acclimatization, use of rest and exercise. *J. Therm Biol.* 6: 79-86.

Pond, C. M. 1984. Physiological and ecological importance of energy storage in the evolution of lactation: evidence for a common pattern of anatomical organization of adipose tissue in mammals. *Symp. Zool. Soc. Lond.* 51: 1-32.

Price, K. (1989). *Territorial Defence and Bequeathal by Red Squirrel Mothers in the Northern Boreal Forest.* M.S. Thesis. University of British Columbia. Vancouver, B. C.

Price, K., K. Broughton, S. Boutin and A. R. E. Sinclair.1986. Territory size and ownership in red squirrels: response to removals. *Can. J. Zool.* 64: 1144-1147.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

Searing, G. F. 1975. *Aggressive Behavior and Population Regulation of Red Squirrels (Tamiasciurus hudsonicus) in Interior Alaska*. M.S. Thesis. University of Alaska. Fairbanks, Alaska.

Schoener, T. W. 1979. Generality of the size-distance relation in models of optimal feeding. *Am. Nat.* 114: 902-914.

Smith, C. C. 1968. *Interspecific Competition in the Genus of Tree Squirrels Tamiasciurus*. Ph. D. Thesis. University of Washington. Seattle.

Sullivan, T. P. and D. S. Sullivan. 1982. Population dynamics and regulation of Douglas squirrel (*Tamiasciurus douglasii*) with supplemental food. *Oecologia* 53: 264-270.

Sullivan, T. P. 1990. Responses of red squirrel (*Tamiasciurus hudsonicus*) populations to supplemental food. *J. Mamm.* 71: 579-590.

Wauters, L. and A. A. Dhondt. 1989. Body weight, longevity, and reproductive success in red squirrels (*Sciurus vulgaris*). *J. of Animal Ecol.* 58: 637-651.

Weiql, P. D. and E. V. Hanson. 1980. Observations on learning and the feeding behaviour of the red squirrel *Tamiasciurus hudsonicus* : The ontogeny of optimization. *Ecology* 61: 213-218.

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Larsen, Karl. Dept. of Zoology. University of Alberta. Edmonton, AB.

Chapter 7

Anticipatory fertility in red squirrels: a search for proximate mechanisms

7.1 Introduction

Red squirrels (*Tamiasciurus hudsonicus*) consume enough cambial tissue of conifers to seriously worry foresters (Sullivan, 1987; Brockley and Elmes, 1987; Rusch and Reeder, 1978). Squirrels also feed on meristematic buds, pollen and seeds of conifers (Rusch and Reeder, 1978). Most authors have assumed that squirrels feed on conifer tissues other than seeds simply to meet energy requirements, particularly when seeds are in short supply (M. Smith, 1968). Kemp and Keith (1970) suggest a more intriguing relationship between squirrels and conifer buds. They propose that in some years fertility in squirrels may be influenced by "superior nutrients" in conifer buds. More specifically, they suggest that the quality of spruce (*Picea sp.*) buds prior to a heavy cone crop enhances fertility of squirrels in advance of the crop. The goal of the present study is to examine potential dietary compounds that might cause anticipatory fertility, and to determine whether red squirrels living in a jack pine (*Pinus banksiana*) forest exhibit increases in fertility in advance of heavy pine cone crops.

The "anticipation" hypothesis was generated to explain years when squirrel fertility was relatively high after a light cone crop, and before a heavy cone crop (Kemp and Keith, 1970). Two other authors, C. Smith (1968) and Kelly (1978) also reported increases in ovulation rates and the proportions of breeding females in seasons following poor cone crops, but preceding large cone crops. Two cases of anticipatory fertility in squirrels were associated with dietary dependence on spruce seeds and buds, whereas C. Smith (1968) noted it in a mixed conifer forest. Because Kemp and Keith (1970) show that winter squirrel populations fluctuate with cone crops over much of Alberta, where the dominant conifers are white spruce (*Picea glauca*), black spruce (*Picea mariana)*, lodgepole pine (*Pinus contorta*), and jack pine, it is of interest to know whether anticipation occurs in squirrels living in pine-dominated habitats.

The anticipation hypothesis is a food-based hypothesis suggesting that some compound(s) in conifer buds enhance ovulatory rate, *inutero* survival and/or the proportion of females breeding, such that more squirrels are produced prior to a high mast crop. There are at least three dietary pathways for this forecasting process. First, plant compounds that enhance FSH and therefore ovulation could be present in conifer buds, and could influence fertility in advance of a large cone crop. Certain plant metabolites are known to increase fertility in microtines (Negus and Pinter, 1966). Growing grass is suspected to contain secondary compounds that trigger reproduction in desert rodents (Reichman, 1975). New herbaceous plant growth is thought to influence ovulation in kangaroos (Newsome, 1966). Such compounds may act as steroids (Labov, 1977; McLachlan *et al.* 1984) or they may be non-steroidal (Butterstein and Schadler, 1988). Perhaps in years prior to a large cone crop, a secondary plant compound is relatively abundant in conifer buds, and it, or its metabolites, enhance puberty and increase ovulation rates in red squirrels.

A second way that fertility could be enhanced by conifer tissues involves the amount of protein in the squirrels' diet. Temperate zone conifers translocate substantial amounts of amino acids to primordial buds before "bud break" (start of the summer growth of needles and maturation of reproductive tissues), so buds may contribute to amino acid requirements during pregnancy (Durzan, 1968; Kramer and Kozlowski, 1979). Rats on protein-deficient diets have higher rates of embryo reabsorption and consequently smaller litters (Nelson &Evans, 1953). If protein limits the number of embryos produced (and consequently litter size), then a dietary increase in protein should boost litter size in squirrels. If this mechanism is valid for pines, litter sizes should vary in an anticipatory fashion: larger average litter sizes should be found in the spring preceding a large jack pine cone crop. Also, squirrels on a high protein diet should have significantly larger litters than squirrels on a normal diet. Finally, conifer buds should comprise a significant part of the red squirrel diet.

A third mechanism for enhancing fertility in squirrels could be that the increase in cone flower buds before heavy cone crops actually provides more caloric energy for squirrels. Territories without sufficient seed energy in some years might, in a "bud" year (Kramer and Kozlowski, 1979), have enough energy to support the costs of reproduction. If this calorie-based mechanism is important, the proportions of females breeding for the first time should be highest prior to a heavy jack pine cone crop (Section 6.2.3).

By definition, (Kemp and Keith, 1970), the spring of 1989 was a jack pine "bud" year because it preceded a relatively large cone crop, and followed two smaller crops. Thus, the proportions of females breeding and litter sizes should be relatively high in red squirrel populations in pine forests during the 1989 breeding season.

7.2 Methods

The Allen Doisy technique (Labov,1977), a bioassay on ovariectomized adult rats, was used to test for plant compounds that could stimulate reproduction through estrogenic activity. Growing jack pine buds collected during the springs of 1989 and 1990 (both years preceded large cone crops) were fed to the ovariectomized rats. If the rats without ovaries exhibited estrus vaginal smears, than the buds would be presumed to contain compounds with steroidal effects.

To test for protein and energy mechanisms, the litter sizes and proportions of squirrels having litters each year were recorded and compared with cone crop production. Sunflower seeds were added to the caches of some female squirrels to determine the effects of high protein on litter size and increased caloric intake on the proportions of females having a litter (6.2.3).

7.2.1 Bioassay for phytoestrogens

To determine whether jack pine buds or sunflower seeds contain phytoestrogens, ovariectomized rats were used in a bioassay (Labov, 1977). Diets containing chemicals that mimic estrogen should cause ovariectomized rats to have vaginal smears similar to normal estrus females with an ovarian source of estrogen. Ovariectomized rats on a standard rat chow diet should show no signs of estrus.

Rats were housed in individual cages in a laboratory maintained by Biosciences Animal Services at the University of Alberta. Twenty rats, fifteen of which were ovariectomized, were divided into four groups of five individuals, which were:

1. Intact normal females on a standard diet of rat chow
2. Ovariectomized females on a standard diet of rat chow
3. Ovariectomized females on a standard diet + buds of jack pine
4. Ovariectomized females on a standard diet + sunflower seeds

Buds of jack pine were collected in April of 1989 and 1990, freeze-dried, ground in a coffee grinder, and mixed with standard rat chow (2 parts buds: 1 part rat chow by volume). For two weeks the rats were allowed to acclimate to their diets. During the following two weeks, vaginal smears were taken from all the rats on a daily basis. Vaginal smears were collected using a cotton swab moistened with saline solution, following a standard technique (Zarrow, 1964). Methylene blue stain was used to dye the smears which were examined under a microscope. Smears were classified by matching them with slides illustrating the reproductive stages in the rat: diestrus, proestrus, estrus and metestrus (Mossman, 1966). The treatment group of a rat was unknown during the smear classification process.

7.2.2 Field observations and food-addition experiments

Patterns of parturition in a red squirrel population were monitored during three breeding seasons (1988-90) in provincial forests south of the village of Fort Assiniboine in central Alberta (N 54° 20', W 114° 50'). The Athabasca Sandhills study site and the squirrel population have been described in previous chapters. Annual weight change (Section 4.3.2), onset of proestrus, timing of estrus and production of litters were recorded for over 100 female red squirrels (Chapter 5). Forty-two other females received renewable caches of sunflower seeds during winter and spring so that the effect of unlimited food on the probability of producing a litter could be monitored (Chapter 6). Annual cone productivity in jack pine was estimated to be greater in 1989 than in 1987 and 1988, as described previously (Section 5.2.2).

Squirrels were trapped in Tomahawk live-traps baited with peanut butter. Once caught, they were transferred to cone-shaped cloth bags, in which weight, identification and reproductive status were determined. When a date of estrus had been determined (Modaferri, 1972), each female was trapped infrequently until her expected date of parturition, at which point trapping was resumed. Sudden loss of weight and lactation signaled that birth had taken place. If the location of the squirrel's nest was not obvious, the lactating female was radio-collared and found with her young. Litter size, sex ratios, litter weight and nest locations were recorded. The number of females producing a litter for the first time (Section 6.3.3) and mean litter size were used to compare relative fertility rates in the population in the three seasons.

Two reproductive classes of adult female squirrels, those who had nursed a litter (multiparous) and those who had not (nulliparous), were determined by nipple condition (Layne, 1954). Actual ages of these females were not known, but by the end of the four year study, it was apparent that the majority of females bred for the first time as two year olds. Cases of primiparity in juveniles (referred to as yearlings in some studies) were very rare (Section 6.3.3). A few squirrels delayed parturition (or even estrus) for two or even three years.

Using focal animal sampling (Altmann, 1974), an estimate of the percentage of time that squirrels spent feeding on buds was determined. For several days in the fall, winter spring and summer, a random selection of up to ten different squirrels were observed. During sampling periods of 40 to 120 minutes, the activity of the squirrel, its distance from its midden (food cache), and its travel distances between scans were noted every 30 seconds. Observations were scheduled so that all daylight hours were sampled in each season. Feeding on buds was one of the activities recorded, permitting a seasonal comparison of the percentage of time spent feeding on buds.

7.3 Results

7.3.1 Phytoestrogen bioassay

The 300 vaginal smears obtained from the ovariectomized rats showed no differences among the treatment and control groups. When five of the ovariectomized rats were injected intramuscularly with 0.1 cc doses of estradiol (Delestrogen (10 mg/cc)), they all produced vaginal smears containing large numbers of squamous epithelial cells typical of normal estrous rats. During post mortem examinations, no differences in uterine vascularization were noted in the remaining animals from the three ovariectomized groups, indicating that they had no exposure to phytoestrogens. In conclusion, there was no evidence that compounds found in jack pine buds or sunflower seeds have any estrogenic properties.

7.3.2 Litter Sizes

In a comparison of the three breeding seasons there was no difference in the mean number of nestlings produced by the wild squirrels (ANOVA, $f_{2,91} = 0.8$, $p = .435$). On average, squirrels had three offspring per litter (Table VII-1). Squirrels given sunflower seeds had slightly larger litters than squirrels on a normal cone diet, but the difference was just barely significant (One tailed t-test d.f. = 118, $p = 0.05$; Table VII-1).

Table VII-1. Litter Sizes in red squirrels

Sample (N)	Mean ± S.D.	Food group
1988 (26)	2.9 ± 0.8	Control
1989 (26)	2.8 ± 0.6	Control
1990 (40)	2.7 ± 0.7	Control
Combined (92)	2.8 ± 0.7	Control (no year effects)
Seed-adds (28)	3.1 ± 0.7	Seed additions (no year effects)

7.3.3 Proportions of squirrels breeding during 1988-90

Variation in the number of primiparous adults in a given year accounted for most of the variation in the proportion of females producing litters. Regardless of weather, fall cone crops (Section 5.2.3) and future cone productivity, parity in adult females with previous breeding experience (multiparous females) remained fairly constant and relatively high each year (Figure VII-1). In 1988 all of the multiparous females in the study (N=32) produced litters successfully. (Six litters were not found, but the mothers continued lactating, indicating that they were still supporting live young. Females that lost litters stopped lactating within a week of losing their young.) In 1989 and 90, an average of only about 13% of the multiparous females failed to breed. Multiparous females given sunflower seeds (N=16) all produced young successfully.

Juveniles (age 10-14 mos.) also showed little variation in the percentage producing a litter from year to year (Figure VII-1). Litters produced by juveniles were uncommon, accounting for less than 10% of all of the parturitions monitored in the 1988-90 seasons. When given sunflower seeds in 1990, eight of 10 juveniles produced litters, suggesting that primiparity in juveniles is mainly limited by energy intake during the spring (Section 6.3.3).

In contrast to the above patterns observed in juveniles and multiparous females, primiparity in adult red squirrels varied much more from year to year. In 1989, a spring prior to a heavy cone crop, only 7% of the nulliparous adults produced young (N=14). In the spring of 1990, 64% of the nulliparous adult female squirrels (N = 25) produced litters for the first time. These results are opposite to expectations for squirrels that anticipate cone crops, because the crops of 1988 and 1990 were smaller relative to the cone production noted in 1989.

Primiparity was greatly increased in adult squirrels by providing excess sunflower seeds at their caches. In 1989, the probability of producing a litter was increased 10-fold in nulliparous females (from 7% to 70%) by the sunflower seed additions. These increases in primiparity were interpreted as the consequence of a decrease in the cost associated with obtaining food energy during the spring (Section 6.4.2).

7.3.4 Feeding on jack pine buds

Observations of squirrel foraging and feeding behavior during fall 1988-fall 1989 indicated that red squirrels include apical buds in their diets year-round. The percentage of

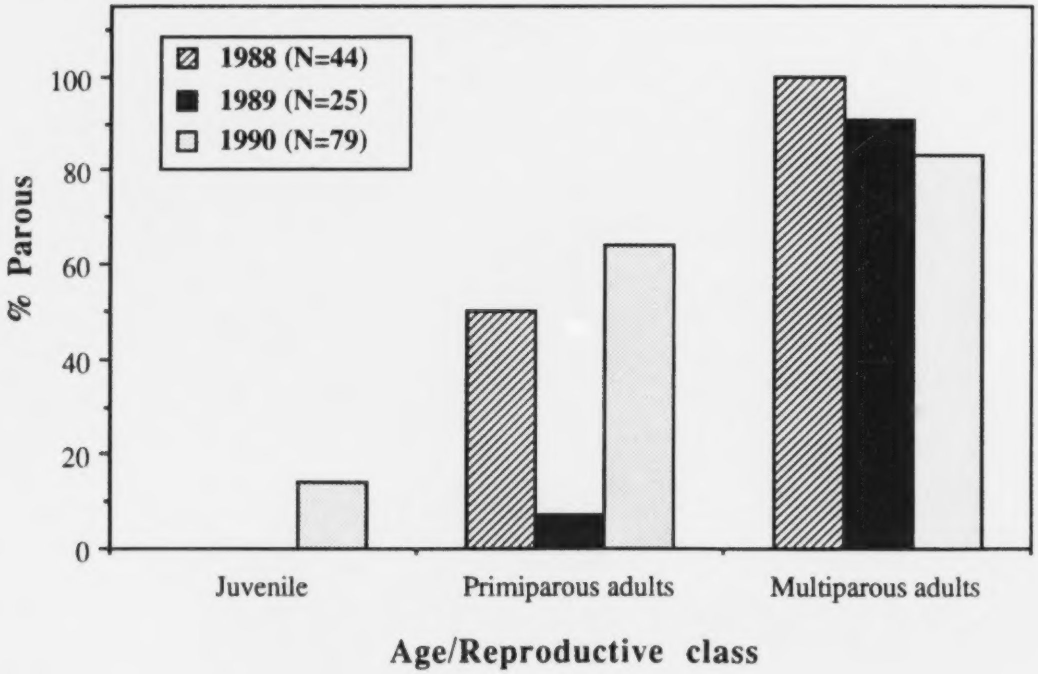

Figure VII-1. Percentage of red squirrel females in different age/reproductive classes that were parous in a given year. (Juvenile litters = 0 in 1989 and were unknown in 1988)

time spent feeding on jack pine buds during the fall and winter was estimated to be almost double the percentage of time spent in the spring (ANOVA $F_{1,73}$ =9.9, p = 0.0001). Feeding on buds was least common in the summer (Figure VII-2). In all 200 hours of systematic observations, the squirrels only consumed the buds that would become needles, or buds of male flowers; flower buds and immature jack pine cones were not consumed.

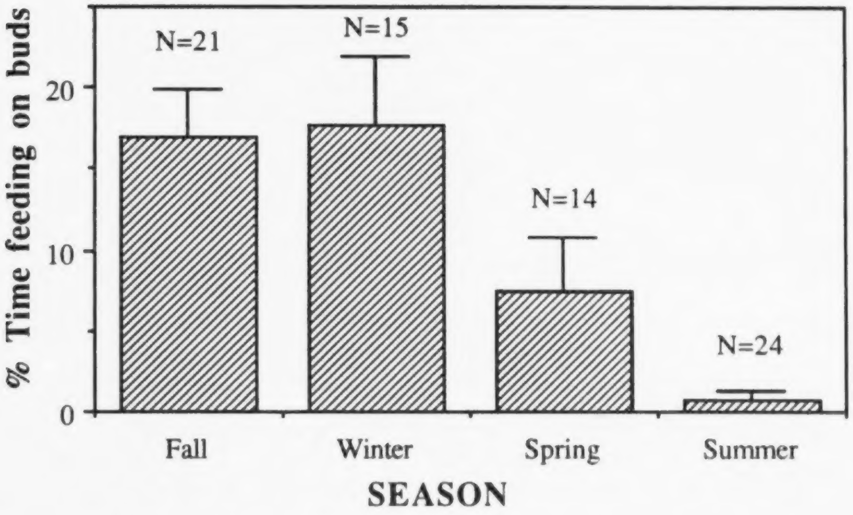

Figure VII-2. Estimated percentage of time spent feeding on buds in different seasons. Females squirrels fed on pine buds year-round, but more time was spent eating them in the fall and winter than in the spring and summer. Sample size (N) refers to the number of focal animal sessions. Standard errors are diagrammed.

7.4 Discussion

C. Smith (1968), Kemp and Keith (1970) and Kelly (1978) had results that evoked the "anticipation hypothesis". In years following light cone crops, but preceding moderately heavy crops, they found evidence for enhanced fertility in squirrel populations. Three potential dietary components of buds: phytoestrogens, excess protein, and excess energy were examined in the present study to determine which, if any, might cause anticipatory fertility.

In the present study, fertility in red squirrels was not enhanced before large jack pine crops. Neither the proportion of females producing a litter nor mean litter size was increased before the relatively heavy jack pine cone crop of 1989. The jack pine cone crop of 1990 may have been larger than the previous crops. This new information, suggests that the increase in the proportion of females breeding in 1990 was anticipatory. However, there was no anticipatory increase in litter size. A relatively mild March and large cone caches at middens (lower foraging requirements) are more likely to have increased the number of breeding females than dietary aspects of pine buds (Section 6. 3.2). These results suggest that anticipatory fertility does not happen in pine habitats. Either jack pine bud nutrients differ significantly from spruce buds, or something other than diet must be responsible for the anticipatory fertility described in the other studies.

7.4.1 Phytoestrogens

Because rodent ovaries are structurally similar (Mossman, 1966), it was assumed that the rat bioassay adequately reflected the results that would have occurred if red squirrels had been used in the experiment. When fed to ovariectomized rats, swollen jack pine buds collected during the spring growth phase (prior to large cone crops) failed to cause estrus vaginal smears in the rats. The spring buds of jack pines do not contain any estrogenic compounds or they were below the threshold for a change in vaginal cytology (Labov, 1977).

Other lines of evidence erode the potential for a phytoestrogens in other conifers. For one, most secondary compounds that effect mammals are produced simultaneously by plants, and as a result, they tend to synchronize reproduction (Sanders, *et al.*, 1981). The timing of estrus in red squirrel populations is asynchronous, sometimes with as much as a two month difference between individuals in the same general location (Rusch and Reeder, 1978; Section 5.3.1). Secondly, fertility in squirrels can be greatly enhanced by feeding squirrels sunflower seeds, which have been shown to be free of phytoestrogens. Finally squirrels kept in the laboratory with no access to buds, but with excess food have larger litters than wild squirrels (this study). These results suggest that anticipatory fertility in red squirrels is not the result of plant secondary compounds (especially estrogenic compounds).

7.4.2 Protein, energy and other potential nutrients

The prediction that protein in jack pine buds preceding a mast crop will enhance fertility in red squirrels was unsupported by field observations. For one, squirrels did not consume the immature cones of jack pine and bud consumption was reduced in the spring relative to winter and fall. Secondly, litter sizes were not largest in the spring prior to the large cone crop of 1989 (or 1990). Likewise, primiparity was not enhanced in the spring of 1989 (although it was in 1990). Thus, in jack pine, dietary components of buds can be eliminated as sources of anticipatory fertility.

The strongest effect of the sunflower seed additions was to increase the proportions of primiparous females in the population. Because caloric content and percentage protein are similar in jack pine and sunflower seeds, it was assumed that increased net energy intake in response to a decrease in handling time was the main cause of the increased primiparity in squirrels given sunflower seeds (Section 2.2.3). Numerous studies have shown that squirrels choose seeds according the highest energy intake per unit time (Gurnell, 1987), further supporting the idea that net energy gain is "biologically perceived" as a major limiting factor by squirrels.

Nevertheless, the results of the sunflower seed feeding experiments suggest that higher rates of protein intake can increase litter size. Litter size was about 11% larger in the females given sunflower seeds than in the control groups. Despite being high in protein, the sunflower seed diet did not achieve the amount of enhanced fertility described by Kemp and Keith (1970) or C. Smith (1968). In years before large cone crops, their litter size estimates increased 40 to 50%. Given that sunflower seeds are more digestible than conifer buds (Gurnell, 1987), some other factor besides dietary protein and energy is needed to explain the great increase in mean annual litter sizes noted in these other studies. Three alternative causes of anticipation are suggested below.

7.4.3 A potential demographic explanation for anticipation

Demography may partially explain population-level patterns of productivity that seem to be anticipatory. In the present study, multiparous adult red squirrels were more productive and less variable in litter production than primiparous females. Adult females tend to shed more ova and produce larger litters than yearlings (Rusch and Reeder, 1978). Thus, the age structure of a red squirrel population can influence the average fertility of a population in a given year.

A result of demography could be interpreted as anticipation. If, in years before a large cone crop, the female population was biased towards adults for some reason (e.g. poor juvenile and yearling survival during the winter), the proportion of lactating females would be relatively large that spring. The larger litters of adults would skew the annual mean litter size upwards in value. Given the age structure of the populations studied by Kemp and

Keith (1970), this demographic alternative is supported by their data. In 1967 their records indicated that breeding-aged females were less abundant than yearlings (1 adult female to 1.5 yearlings); and this was the year of low productivity. In contrast, in 1968, the year with anticipatory fertility, the number of breeding-aged adults in the population was 1.5 times greater than the number of yearlings.

The populations of squirrels studied by Kelly (1978) and C. Smith (1968) were also potentially skewed towards breeding adults prior to the peak cone crop. Juvenile mortality is higher than adult mortality (Kemp and Keith, 1970; Rusch and Reeder, 1978), so after a year or two of poor cone production or extremely cold winters and springs, a squirrel population would probably be biased towards the hardiest of reproductive adults.

7.4.4 Spring temperatures and anticipatory fertility

When one examines the mean monthly temperatures recorded for Rochester, Alberta, the spring of 1967 was colder than the 1968 spring when the anticipatory fertility occurred. February and March of 1967 were colder than the ten year average for the 1960's, whereas the same months in 1968 were warmer than the ten year average (Climate Services, University of Alberta). Given that cone crops in the previous falls (1966 and 1967) were about the same (Rusch and Reeder, 1978), one would still expect to find a substantial increase in the proportion of females breeding in 1968 relative to 1967, because of the better bioenergetic conditions. The fact that the start of breeding season in 1968 was about two months earlier than in 1967 (Rusch and Reeder, 1978) further supports the assumption that the milder spring of 1968 affected squirrel reproduction (Chapter 5).

7.4.5 Potential dietary reason that anticipation occurs in spruce but not in pine habitats.

Setting the demography and temperature arguments aside, there is some evidence that "bud" years could alter the dietary quality of squirrels in spruce but not in pine habitats. Although data were not collected to address the issue of nutritional differences between jack pine and black or white spruce buds *per se,* pertinent data are available (Gurnell, 1987) comparing the quality of buds and seeds of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*).

The energy (18-22 kJ/g), handling time and digestibility of conifer buds is similar across many species of trees (Gurnell, 1987), so variation in bud energy is not a likely source of the difference between spruces and pines. Because spruce buds contain less energy (approx. 21 kJ/g; M. Smith, 1968) than spruce seeds (25 to 28 kJ/g; Gurnell, 1987) and are less digestible, energy in buds is a very unlikely cause of anticipatory fertility in spruce, or any conifer habitat for that matter.

When one examines the percentages of soluble protein and minerals in buds and seeds, spruce differs substantially from pine. In Norway spruce, protein and phosphorus levels are higher in buds than seeds, but the opposite relationship holds in Scots pine (Gurnell, 1987). Thus in a year of seed scarcity (a "bud" year), squirrels feeding on pine buds experience a decrease in protein and phosphorus, while squirrels feeding on spruce buds consume higher levels of phosphorus and protein than usual (Table VII-2). When digestibility is assumed to be 75% for buds and 92% for seeds, spruce seed has a lower protein content (15.7%, Gurnell, 1987) than sunflower seed (20.6%, Gebhardt & Mathews, 1981), jack pine seed (20.8%; this study, Appendix II), and Scots pine seed (25%, Gurnell, 1987). In contrast, when squirrels consume mainly spruce buds they obtain 22.2% protein, whereas a pine bud diet provides only 12.8% protein (Gurnell, 1987).

There are no other obvious dietary constituents besides protein and phosphorus that could cause anticipation in spruce without the same effect in pine (Table VII-2). Because phosphorus levels in sunflower seeds are the same as spruce, protein levels may be singled out as the most likely source of anticipatory litter sizes. In conclusion, it seems possible that squirrels are limited by protein when they rely on a spruce seed diet, but not when they rely on a pine or sunflower seed diet, or include spruce buds in their diet. Thus, in a spruce "bud" year, ovulation rates and litter sizes would be expected to increase. The proportions of females having litters would not be expected to increase because food energy would be less available. Thus, it seems impossible for dietary aspects of spruce buds to be solely responsible for anticipatory fertility. Increases in the proportions of females breeding, ovulation rates and litter sizes can all result in a spring when the squirrel population is biased towards adults. Thus, the age structure argument is a least more parsimonious than the bud nutrients hypothesis.

This study suggests that interactions between temperature, food availability and quality, and the age structure of a population are likely to be shaping population-level productivity in ways we have not fully considered. More study is needed to determine whether anticipatory litter sizes in spruce result mainly from bud quality, or from non-dietary processes.

Table VII-2: Qualitative energy and nutrient changes in Scots pine and Norway spruce depending on whether the diet is mainly buds or seeds. Evaluation is based on Table 3.1 of Gurnell (1987). (D = decrease, I = increase, 0 = no change)

	Cone shortage = diet mainly buds		No cone shortage = diet mainly seeds	
Item	Pine	Spruce	Pine	Spruce
Energy	D	D	I	I
% protein	D	I	I	D
Sodium	0	0	0	0
Potassium	I	I	D	D
Calcium	I	I	D	D
Magnesium	D	0	I	0
Phosphorus	D	I	I	D

7.5 Literature Cited

Altmann, J. 1974. Observational Study of Behavior: Sampling Methods. *Behavior* 49: 227-267.

Brockley, R. P. and E. Elmes. 1987. Barking damage by red squirrels in juvenile-spaced lodgepole stands in south-central British Columbia. *For. Chron.* 63: 28-31.

Durzan, D. J. 1968. Nitrogen metabolism of Picea glauca. I. Seasonal changes of free amino acids in buds, shoot apices, and leaves, and metabolism of uniformly labelled ^{14}C-L-arginine by buds during the onset of dormancy. *Can. J. Bot.* 46: 909-19.

Gurnell, J. 1987. *The Natural History of Squirrels*. Christopher Helm, London.

Kelly, D. G. 1978. Population Density, Territoriality and Foraging Ecology of Red Squirrels (*Tamiasciurus hudsonicus*) in Black and White Spruce Forests of Interior Alaska. M.S. Thesis. University of Alaska, Fairbanks, Alaska.

Kemp, G. A. and L. B. Keith. 1970. Dynamics and regulation of red squirrel (*Tamiasciurus hudsonicus*) populations. *Ecology* 51(5): 763-779.

Kramer, P. J. and T. T. Kozlowski. 1979. *Physiology of Woody Plants*. Academic Press, New York.

Labov, J. B. 1977. Phytoestrogens and mammalian reproduction. *Comp. Biochem. Physiol.* 57(A): 3-9.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Layne, J. N. 1954. The biology of the red squirrel, *Tamiasciurus hudsonicus* (Bangs), in central New York. *Ecol. Monogr.* 24: 227-267.

Modafferi, M. M. 1972. Aspects of Reproductive Biology of the Red Squirrel (*Tamiasciurus hudsonicus*). M.S. Thesis. University of Alaska. Fairbanks, Alaska.

Mossman, H. W. 1966. The rodent ovary. In: Comparative Biology of Reproduction in Mammals. *Symposia of the Zoological Society of London #15*. London.

Negus, N. C. and A. J. Pinter. 1966. Reproductive responses of Microtus montanus to plants and plant extracts in the diet. *J. Mammal* 47: 596-601.

Nelson , M. M. and H. M. Evans. 1953. Relation of dietary protein levels to reproduction in the rat. *J. Nutrit.* 51:71-84.

Newsome, A. E. 1966. The influence of food on breeding in wild kangaroo in Central Australia. *C.S.I.R.O. Wildl. Res.* 11:187-196.

Pond, C. M. 1984. Physiological and ecological importance of energy storage in the evolution of lactation: evidence for a common pattern of anatomical organization of adipose tissue in mammals. *Symp. Zool. Soc. Lond.* 51: 1-32.

Reichmann, O. R. and K. M. Van De Graff. 1975. Association between ingestion of green vegetation and desert rodent reproduction. *J. Mammal* 56: 503-506.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

Sanders, E. H. , P. D. Gardner, P. J. Berger, and N. C. Negus. 1981. 6-Methoxybenzoxalinone: a plant derivative that stimulates reproduction in *Microtus montanus*. *Science* 214: 67-70.

Smith, M. C. 1968. Red squirrel responses to spruce cone failure in interior Alaska. *J. Wildl. Mgmt.* 32(2): 305-317.

Sullivan, T. P. and A. Vyse 1987. Impact of red squirrel feeding damage on stands of lodgepole pine in the Cariboo Region of British Columbia. *Can. J. For. Res.* 17: 666-674.

Zarrow, M.X. , J.M. Yochim, and J. L. McCarthy. 1964. *Experimental Endocrinology: a source book of basic techniques*. Academic Press. New York.

Chapter 8

Concluding Remarks

8.1 Summary of Results

Like humanity's dependence on a few types of grain, much of our excellent information on proximate control of mammalian reproduction comes from only a handful of species (Bronson, 1989). During the past 25 years, researchers of red squirrel populations have made frequent reference to our incomplete understanding of the proximate factors that determine the breeding schedule and variation in productivity in this species (Lair, 1985; Rusch and Reeder, 1978; Kemp and Keith, 1970; C. Smith, 1968; M. Smith, 1968). These paradigm gaps, along with the research interests of my advisors, seeded this Ph. D. study on the proximate factors influencing the timing and occurrence of reproduction in red squirrels.

The broad objectives of the study were:

1. To determine the proximate annual cues of proestrus and estrus in the red squirrel,
2. To formulate and test hypotheses about environmental factors that may cause variation in timing of estrus and parturition in squirrels, and
3. To determine the proximate factors that cause variation in fertility, especially rates of primiparity in red squirrels.

These goals have been met, and the results give a holistic, but preliminary picture of the proximate intrinsic and extrinsic variables that influence reproduction in red squirrels living in boreal conifer forests. As listed in Tables VIII-1 and VIII-2, some of the potential factors that were thought to affect the timing and occurrence of reproductive readiness, estrus and parturition in female squirrels have been verified as doing so. Many are interdependent. A few variables can be eliminated because they seem to have no influence on reproduction in squirrels. Others may require further study.

Table VIII-1. Potential non-social factors that influence or fail to influence timing of reproduction in wild red squirrels. (R=rejected as a critical factor ; A=Accepted as a critical factor or cannot be rejected)

Extrinsic Factors	Comment on importance of the factor
Photic conditions	A, Affects timing of proestrus
Phytoestrogen cue	R, Absent in pine buds, no evidence for them
Nutrient cues*	R, No evidence for influence on timing
Bare ground	R, Unimportant, spurious correlation with temperature
Cone Crop size	A, Affects net energy gain, but not as much as temperature
Temperature	A, Extremely important for timing net energy gain
Food type	A, Energy per unit handling time affects timing
Intrinsic Factors	**Comment on importance of the factor**
Individual's sensitivity to net energy gain	A, Potential mechanism for timing estrus
Age	A, Influences foraging behavior linked to net energy gain
Body fat gains in spring	A, Associated physical trait of net energy gain
Behavior	A, Caching, cone-stripping, foraging stategies important
Body size in spring	R, Not important during breeding season
Winter weight loss	R, Not critical if squirrel survives winter

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* Nutrient = dietary needs other than energy.

Table VIII-2. Potential non-social factors that influence or fail to influence the probability of reproduction in wild red squirrels. (R=rejected as a critical factor ; A=Accepted as a critical factor or can not be rejected)

Extrinsic Factors	Comment on importance of the factor
Photic conditions	R, Do not seem to prevent or permit female reproduction
Phytoestrogens	R, Absent in pine buds, not required, no evidence for them
Nutrient requirements	A, Ovulation rates and litter sizes affected by protein
Bare ground	R, Unimportant, spurious correlation with temperature
Cone Crop size	A, Influences net energy gain
Temperature	A, Extremely important; influences net energy gain
Food type	A, Energy per unit handling time critical
Territory quality	A, Size and density of trees near midden have import
Territory Size	R, Not critical for presence or absence of parturition

Intrinsic Factors	Comment on importance of the factor
Individual's sensitivity to net energy gain	A, Likely cause of failure to breed in some years
Age	A, Extremely important because of influence on behavior
Body fat stores in spring	A, Probability of reproduction positively correlated
Behavior	A, Caching, cone-stripping, foraging stategies important
Body size in spring	R, Not important during breeding season
Winter weight loss	R, Not critical if squirrel gains weight in spring

In reference to Table VIII-1, experimental results indicate that photoperiod can play a role in timing the onset of proestrus in red squirrels. Reproductive readiness in female squirrels was cued and advanced by exposure to increasing day lengths and retarded by exposure to short day conditions (Chapter 3). However, continual exposure to short days did not prevent proestrus or estrus in all females. These results suggest that (1) endogenous rhythms are entrained by day length (Section 3.4), (2) some individuals remain responsive to light cues, but (3) high melatonin levels presumed in short day experiments, are not capable of completely supressing reproduction in some squirrels.

As a result of laboratory studies with light, there were as many unanswered questions as there were highly probable answers:

1. Is there a genetic basis for the apparent variation in sensitivity to photoperiod?
2. Could juvenile squirrels be entrained to breed in a different season in the lab?
3. Are male red squirrels photosensitive or not? When ?

Because the timing of reproductive readiness is entrained by increasing photoperiod, female squirrels begin their seasonal reproductive effort before the spring and summer seasons when temperatures and food availability permit optimal survival of the mother and her litter. Still, squirrels should not rely on photic cues alone. In years with extremely long winters (cold springs), reliance on increasing photoperiod to time reproduction would likely result in a reproductive effort made too early in the season to succeed. Thus, other proximate mechanisms are needed to prevent early breeding. By evolving net energy gain requirements or a body fat threshold for timing estrus, red squirrels seem to have solved this problem.

Individual sensitivity to caloric balances and body condition (fat levels) after proestrus play a critical role in timing ovulation and parturition (Chapters 5 & 6). Females may require different levels of body fat to trigger ovulation. The evidence in support of individual variation in body fat or net energy gain requirements includes (1) the substantial asynchrony in estrus dates exhibited by squirrels when food was equally abundant, (2) the association of early weight gain with early estrus dates, and (3) the tendency for fat squirrels to breed later in the season.

Because squirrels harvest cones and fatten in the late fall, they cannot depend on body condition alone as a proximate cue for reproduction. For red squirrels and probably many other boreal mammals, day length and body condition interact as proximate cues that ensure that the timing of reproduction can be modified within a season, but is restricted to a larger block of time.

A host of intrinsic and extrinsic factors further influence net energy gains achieved by individual squirrels each spring. Age, territory quality and behavior affect body weight and the probability of producing a litter. Over-winter growth in juveniles competes with reproduction for energy during the spring, and in jack pine appears to be one reason that most females fail to ovulate or produce a litter in the breeding season following their birth. Now that it is clear that age and net energy gain are strongly interrelated, further studies on the ontogeny of foraging, caching, and food handling behavior in squirrels are needed. Some behaviors like the stripping of cones are especially interesting.

Preliminary studies have eliminated some potential age-related differences in behavior. For one, the number of cones cached in the fall do not differ between older (multiparous) and younger (nulliparous) females (Zimmerling, pers. comm). In addition, the seed calories contained in the different ages of jack pine cones show no difference (this study, Appendix II). Age seems to affect the cone harvesting strategies of females (Boonstra, pers. comm.; see Section 6.4.3) and this pattern deserves more study. Time budget analysis and a measure of metabolic costs as a function of age could begin to quantify relationships between behavior and net energy gain that are suspected to preclude many young animals from breeding.

8.1.1 Conclusions about anticipatory fertility

Squirrel productivity in jack pine did not confirm Kemp and Keith's (1970) anticipation pattern. Mean annual litter size did not show an increase in the breeding seasons before large cone crops (1989 and 1990). This may have been because the dietary proximate factors that influence productivity in pine differ from those in spruce, or because anticipatory fertility is not caused by conifer bud nutrients (Chapter 7). Now that three alternative mechanisms for anticipatory fertility have been outlined (age structure of the female population, spring temperatures, and protein levels in the diet), it would be interesting to test them rigorously in spruce and other conifer habitats. The age structure of territorial squirrels could be altered by trading adults established on territories with established yearlings during the fall, and then monitoring productivity differences as a function of age structure. Protein in the diets of individuals could be enhanced artificially, and consequent litter sizes monitored. Squirrel researchers with a good knowledge of the age structure of their study population, records of the timing of mast cone crops, and annual spring temperatures may have the data to test for these mechanisms without having to collect new information.

8.2 Conclusion

Female red squirrels are not the epitome of Negus and Berger's (1987) "facultative" breeder, nor do they fit the model of an "obligate" breeder. Rather than having obligate responses to environmental predictors, their reproduction is dependent on a suite of environmental factors: day length, spring temperatures, and fall cone crops preceding the breeding season.

Nevertheless, it is clear from this study that reproduction in red squirrels is not opportunistic. Timing of reproductive events was only moderately changed by experimental conditions (i.e. photoperiod studies - Chapter 3; seed addition - Chapters 5 & 6). Observable responses to photoperiod were for the most part restricted to the time of year when day length would be increasing naturally, showing that endogenous rhythms of reproduction appropriate for the boreal environment are well-established in *Tamiasciurus hudsonicus* populations. Unlike findings for many small, opportunistic rodents (Bronson, 1989), excess food did not cause winter breeding in red squirrels. Net energy gain seems to interact with an endogenous cycle of reproductive readiness entrained by day length to prevent opportunistic breeding, and to provide a level of finer-tuning for timing estrus and parturition in spring.

During each breeding season, red squirrel natality is constrained by proximate environmental conditions. Fundamental adaptations exist in this species that prevent the waste of reproductive energy. Perhaps, as a result of saving this energy, each squirrel that fails to breed has a high probability of living through another boreal winter. In the following spring its own rather strict criteria for reproductive investment will be tested all over again. With such strict sensitivity to energy for reproduction, short life is not an option for reproductive success in boreal red squirrels. Reproductive constraint is a correlate of long life and enough learning capacity to permit improvement in foraging efficiency during an individual squirrel's life.

Prior to this study, autumn mortalities, especially of dispersing juveniles were cited as the major factor regulating populations of red squirrels (Gurnell, 1987). There is no doubt that density-dependent regulation occurs in the autumn when intraspecific competition for territories is high. Nevertheless, this study makes it clear that a great deal of population regulation is not related to fall densities, but is related to constraints on reproduction that occur during the spring. At this time, natality is fine-tuned by food availability and temperature and the individual squirrel's response to these conditions. Thus, both proximate and ultimate processes act in concert to select the genotypes and phenotypes of North American red squirrels.

8.3 Literature Cited

Bronson, F. H. 1989. *Mammalian Reproductive Biology*. University of Chicago Press, Chicago.

Gurnell, J. 1987. *The Natural History of Squirrels*. Christopher Helm, London.

Kemp, G. A. and L. B. Keith. 1970. Dynamics and regulation of red squirrel (*Tamiasciurus hudsonicus*) populations. *Ecology* 51(5): 763-779.

Lair, H. 1985. Mating seasons and fertility of red squirrels in southern Quebec. *Can. J. Zool.* 63: 2323-2327.

Negus, N. C. and P. J. Berger. 1987. Mammalian reproductive physiology: adaptive responses to changing environments. In: *Current Mammalogy*. New York, Plenum Press. 149-173.

Smith, C. C. 1968. The adaptive nature of social organization in the genus of tree squirrels *Tamiasciurus*. *Ecol. Monogr.* 38(1): 31-63.

Smith, M. C. 1968. Red squirrel responses to spruce cone failure in interior Alaska. *J. Wildl. Mgmt.* 32(2): 305-317.

Rusch, D. A. and W. G. Reeder. 1978. Population ecology of Alberta red squirrels. *Ecology* 59: 400-420.

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Appendix I. 1988 Feasibility of captive breeding in red squirrels

Six female and seven male squirrels were brought into captivity as of January 2, 1988. The squirrels were placed in two rooms. In one room they were kept in separate cages 40x40x60cm. In the other room (3x4x3m) squirrels were free to climb on branches and rocks spead about room. Females and males had physical contact in the social setting, but only nontactile (pheromonal) interactions were possible among squirrels in the cages.

In both rooms, the lights were kept at 12 hours of light and dark. The main diet of all the squirrels was a combination of laboratory rat chow and sunflower seeds.

Each week, weight, vaginal width, scrotal length, nipple condition, vaginal smear conditions and anal gland activity of the squirrels were recorded.

Three females came into estrus, two in the social room and one in an isolated cage. All had bred previously (multiparous). The other females showed no sign of a complete estrus cycle, although one had vulva swellings indicative of proestrus. These females had no breeding experience prior to captivity (nulliparous). The females that cycled in the social room bred successfully and had litters. One female bore five young and the other had four.

The squirrels bred when the light to dark ratio was 12:12 hours. Breeding in wild squirrels monitored in the field occurred before the captives despite of the fact that the captive squirrels had all the food they could eat. The two captive squirrels bred synchronously, with copulations noted on May 3-5. Births were on June 10 and 11 suggesting a gestation period of 36-39 days.

Unlike wild squirrels, captive female squirrels had no anal gland swelling, suggesting that the production of anal gland exudate was not promoted under captive conditions. However, the same effect was not noted for males. Male anal gland activity was similar to that observed in wild squirrels.

When males were caught in January, four were scrotal and three were inguinal. All became scrotal by February 10. Testes size was greatest during April. Regression began in late May and all males were inguinal again by early July. Two became inguinal by mid-June.

In conclusion, the most important finding of the feasibility study was that females will cycle and breed in captivity. Thus, experimental conditions were established to monitor effects of light and nutrients on estrous cycles of red squirrels. Because the three squirrels that failed to have normal estrous cycles were all nulliparous, only multiparous females were used in the experiments that followed the feasibility study. To prevent obesity, all captive squirrels were given the opportunity to exercise. Some of the caged squirrels in the feasibility study became extremely obese, gaining 50 to 100 grams in two weeks.

Appendix II. Calories, lipid and protein contents of jack pine seed and mixed fungi

A. Methods and results of analyses done November 1987:

Jack pine seeds:

To open jack pine cones, the cones were boiled for 2 minutes, and then baked in a kitchen oven at 150°F for 2 h. Seeds were removed from the cones, counted and weighed. From 307 cones, 48g of seed plus the seed coat were obtained. A mean of 32.6 ± 12 seeds per cone were noted for this sample. In this first bomb calorimetry, both jack pine seeds, seed coats and their "wings" were included. The seeds and wings were ground in a 1KA-WERK A-10 grinder until homogeneous. The mixture was dried over night. Two aliquots of about 2 grams of the mixture were placed in small aluminum drying pans, weighed and placed in a 60°C drying oven. Twelve hours later the two samples were weighed again to determine the percentage of dry matter in the seeds. The remainder of the dried mixture was split into duplicate samples for bomb calorimetry, Kjeldahl nitrogen determination, and Goldfish crude fat analysis. These procedures are standard and referenced in Chapter 2.

Mushrooms:

The same analyses were done on dried, ground fungi that were found on the middens belonging to squirrels. The fungi consisted of a mixture of gill and "boletus" mushrooms.

Results of squirrel food analyses, 1987 (all results from duplicates; N=2):

Food item	% dry matter	cal/g	% protein	% fat
Jack pine seed + wings	93.56 ± 0.17	5479 ± 1.3	21.14 ± 0.18	26.4 ± 0.14
Assorted mushrooms	77.0 ± 0.30	4750 ± 9.4	17.8 ± 0.10	NA

B. In the fall of 1990, T. Zimmerling and L. Boonstra were trying to understand why squirrels preferred to harvest green jack pine cones rather than older cones. They thought there might be a caloric difference in the seeds from these cones, so we did 12 bomb calorimetry tests, four samples each of green, gold and grey cones (as cones age they change color). Only the seed and its seed coat were included in the sample. This still resulted in an underestimate of caloric intake by the squirrel, because the squirrels remove both the wing and the seed coat, and eat only the endosperm.

The same procedures described above in section A were used to extract, dry, and determine caloric content of the seeds. Only caloric contents of the seeds were determined. The mean caloric content from the 12 seed samples was 6119 ± 63 cal/g.

Results of seed analyses April, 1990

Cone color	(N)	No. seeds/cone	Seed biomass (g)/cone	% dry matter	cal/g
Green	15	37 ± 8.8	0.16 ± .06	96.23	6084 ± 88
Gold	15	35 ± 11	0.14 ± .06	96.79	6134 ± 41
Grey	15	41 ± 17	0.19 ± .07	96.8	6138 ± 55